17 September 2004

Science

Vol. 305 No. 5691
Pages 1661–1856 $10

INSIDE NORTH KOREAN SCIENCE

AAAS

Science

COVER Pohyon Temple, north of Pyongyang, and tissue culture experiments by Un Song Gun (inset) illustrate North Korea's ancient roots and scientific hopes. Its leaders are quietly encouraging scientists to seek foreign collaborations and funds. A special News Focus on science in North Korea begins on page 1696; see also the Editorial on page 1677. [Photos: Richard Stone]

Volume 305
17 September 2004
Number 5691

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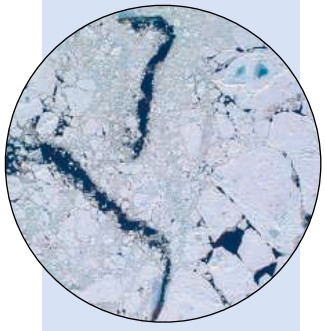

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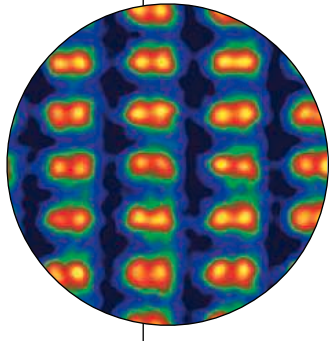

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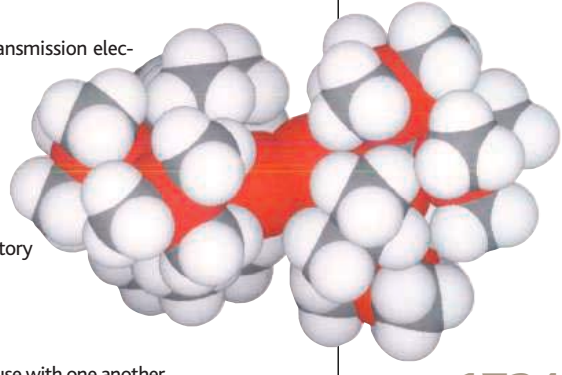

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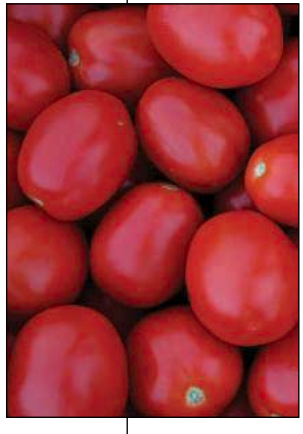

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SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Domestic individual membership and subscription (51 issues): $130 ($74 allocated to subscription). Domestic institutional subscription (51 issues): $500; Foreign postage extra: Mexico, Caribbean (surface mail) $55; other countries (air assist delivery) $85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. **Printed in the U.S.A.**

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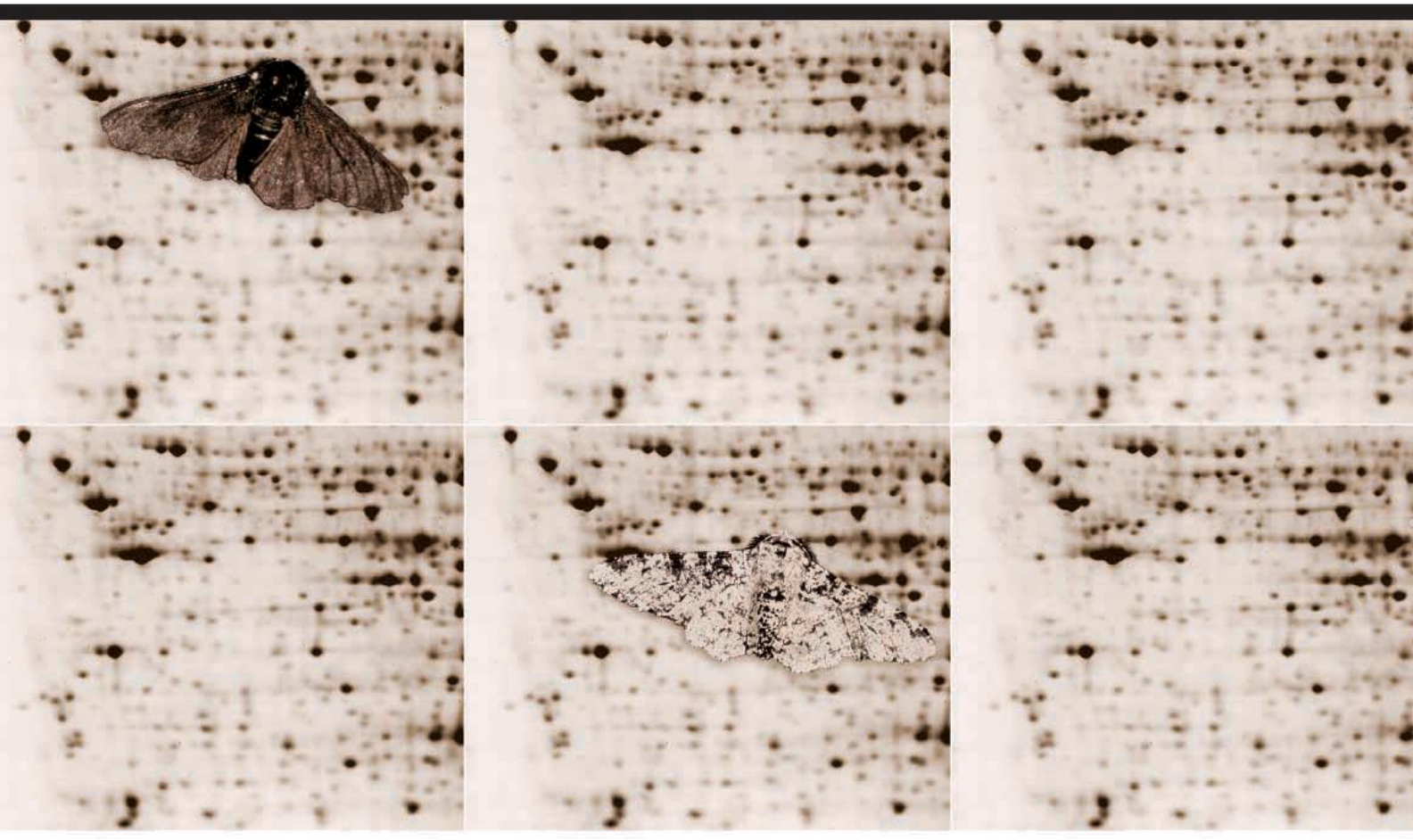

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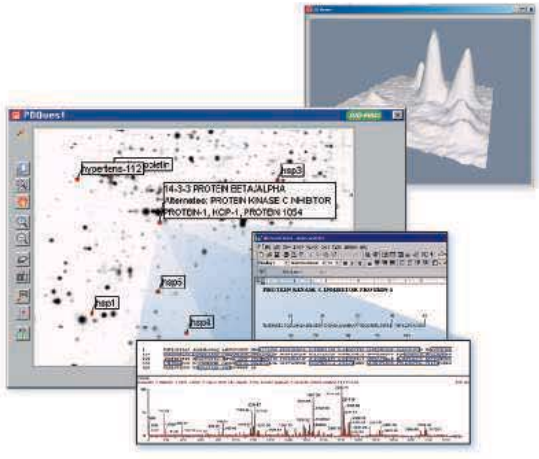

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Exoplanet Says Cheese

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Stroke victim helps researchers locate brain's dream center.

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MiSciNet: Aspirations of a Singing Doctor *E. Francisco*

Cosmo Fraser's first love was math, but this extraordinary scientist now takes care of patients, teaching, and music.

MiSciNet: Starting Graduate School—Mathematics Training, Part 2 *C. Castillo-Chavez*

Read more advice to students interested in math about that critical first year of graduate studies.

Global/Canada: Navigating by the Numbers *A. Fazekas*

A University of Calgary expert tells how software plays a key role in interpreting global positioning data and is used to integrate, manipulate, and display a wide range of information.

UK: A Transferable Skills Toolkit for Postdocs *P. Dee*

Phil Dee unveils the hidden transferable skills that postdocs, by default, have acquired.

UK: Dead-End in Academia—Redundancy with No Lectureship Ahead *M. O'Neill*

Now 12 years on in academia, Mary O'Neill faces redundancy from her postdoc position and wonders what happened to her once brilliant science career.

Netherlands: Europe Chooses "World Leaders of the Future" *H. Obbink*

Hanne Obbink talks to one of the Dutch winners of the European Young Investigators Awards [in Dutch].

science's sage ke www.sageke.org Science of Aging Knowledge Environment

Perspective: A Century of Population Aging in Germany *E. Hoffmann and S. Menning*

How old is Germany?

News Focus: Tarnished Vision *R. J. Davenport*

Iron glut clouds eyes in mice.

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Disciplining misshapen proteins leaves cells vulnerable to oxidative stress and death.

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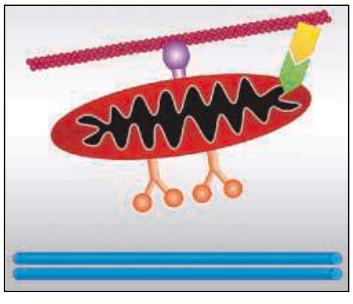

Moving mitochondria in axons.

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Perspective: Emerging Role for ERK as a Key Regulator of Neuronal Apoptosis *E. C. C. Cheung and R. S. Slack*

Kinases better known for regulating growth and survival turn deadly in a model of neuronal cell death.

Perspective: Mitochondrial Stop and Go—Signals That Regulate Organelle Movement *I. J. Reynolds and G. L. Rintoul*

Does NGF signal a mitochondrial docking station on the "microtubule railroad"?

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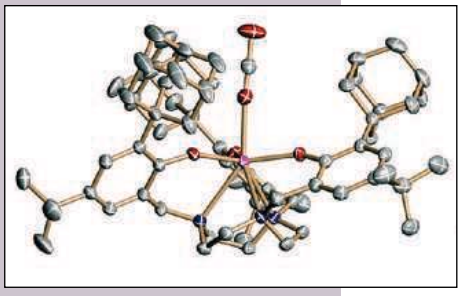
Sugar in Two Steps

Hexose sugars are naturally abundant, but it is often useful to modify their structures for chemical and biochemical studies. Standard synthetic routes tend to be long and tedious and require multiple protection steps. **Northrup and MacMillan** (p. 1752, published online 12 August 2004) now describe a reaction sequence for generating the sugars from achiral aldehyde precursors in just two steps, thereby offering a convenient means of preparing diverse structural variants. In the first step, α-oxyaldehydes are dimerized with L-proline as the only source of asymmetry throughout the sequence. In the second step, an aldol addition-cyclization step is controlled by variation of solvent and Lewis acid to afford any of three stereoisomeric products (glucose, mannose, or allose), all in high yield and stereochemical purity.

Standing CO_2 on Its End

Understanding how plants reduce CO_2 to sugars, and facilitating attempts to mimic this chemistry, requires better insight into the specific binding geometry of CO_2 at metal centers. Synthetic chemists studying the problem usually start with metal complexes that coordinate CO_2 through the C atom, with one or both O atoms bent away from the metal. **Castro-Rodriguez *et al.*** (p. 1757) have prepared a U complex in which coordinated CO_2 remains linear and binds end-on to the metal through a single O atom. X-ray crystallography verified this unusual bonding geometry.



Disilyne Debut

Double and triple bonds are common in compounds of the first-row elements carbon, nitrogen, and oxygen. In contrast, the heavier main group congeners tend to form single-bonded networks instead, because repulsion by inner-shell electrons keeps the atoms too far apart for π-bonding. **Sekiguchi *et al.*** (p. 1755; see the Perspective by **West**) have managed to push two Si atoms close enough together to form a Si-Si triple bond. They reduced a brominated precursor in which the Si atoms bear very bulky side groups that help destabilize more conventional bonding options. X-ray crystallography revealed a bent geometry consistent with theoretical predictions that the silicon orbitals do not hybridize like those of carbon do in rigidly linear alkynes.

Early Oxygen History

Measurements of the three stable isotopes of oxygen in primitive meteorites that formed in the solar nebula indicate that the nebular gas had an initial enrichment in ^{16}O that was quickly depleted. Observations of molecular clouds indicate that ultraviolet radiation selectively dissociates $C^{17}O$ and $C^{18}O$, but not $C^{16}O$, which leaves the atomic oxygen gas in the interior of the cloud depleted in ^{16}O. **Yurimoto and Kuramoto** (p. 1763; see the Perspective by **Yin**) have developed a model to explain the meteoritical data using the astronomical observations. The oxygen isotopic differences developed in the molecular cloud via photodissociation. When the cloud collapsed into the solar nebula disk, the isotopic differences were transported to the inner disk by icy dust grains that evaporated when they neared the Sun.

Why the Ice?

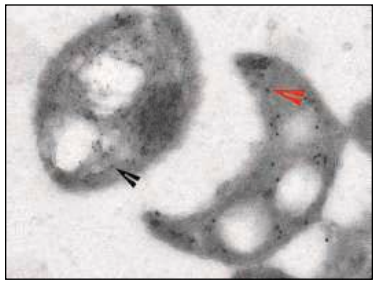
The large, permanent ice sheets that presently occupy Antarctica began to form around 14 million years ago, when Earth entered a phase of global cooling. However, the climate processes that produced these changes, as well as the temporal relation between ice sheet growth and cooling, have remained obscure. **Shevenell *et al.*** (p. 1766) analyzed Mg/Ca ratios (a proxy for temperature), oxygen isotopes (which record a combination of temperature and seawater oxygen isotopic composition), and carbon isotopes (a proxy for atmospheric CO_2 concentrations) of benthic foraminifera from Southern Hemisphere marine sediments with ages between 15 and 13.2 million years. Deep-ocean cooling began roughly 60,000 years before ice sheet growth, and both of these processes happened during a period of atmospheric CO_2 increase. These findings suggest that factors other than radiative forcing, such as ocean heat transport, were key elements of this climate transition.

Damage-Free Dating

Many geologic boundaries reflect dramatic changes in species abundances or mark the origination of species. Thus, the accurate determination of their ages is essential for defining the pace of evolution. One of the best dating methods, based on the decay of U isotopes to Pb can be problematic if damaged parts of zircons, the primary uranium-bearing mineral, lose radiogenic Pb or incorporate older cores. **Mundil *et al.*** (p. 1760; see the News story by **Kerr**) used a recent method that strips out these damaged areas to refine the age of the end-Permian extinction and Permo-Triassic boundary. Their data on a sequence of ashes in two localities place the extinction at 252.6 million years ago, about 1 million years older than previously determined. The results support the conclusion that the extinction occurred within the limit of the method, just a few hundred thousand years.

Two Membranes, Two Fusion Mechanisms

Mitochondria, the powerhouses of the cell, are surrounded by a double membrane. Within the cell, mitochondria continually fuse with one another, but the mechanism by which their two membranes can faithfully fuse remains obscure. **Meeusen *et al.*** (p. 1747, published online 5 August 2004; see the Perspective by **Pfanner *et al.***) now present a cell-free assay that reconstitutes efficient mitochondrial fusion in



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vitro. In the assay, the fusion of the outer and inner mitochondrial membranes can be individually scrutinized, and the two fusion events can be mechanistically distinguished.

Lasting Legacy of Formative Years

Development and disease susceptibility are not purely a function of genotype—environment plays a large part in shaping an organism and in its demise. Furthermore, the environment begins having its effect at the earliest of stages of development, during periconception, fetal, and infant stages. The concept of developmental origins of disease has gained credence through epidemiological and clinical studies. **Gluckman and Hanson** (p. 1733) review fundamental observations, discuss mechanisms of action, and discuss the concept of developmental origins of disease from an evolutionary perspective. **Finch and Crimmins** (p. 1736) suggest that exposure to infection and other environmental sources of inflammation during infancy and childhood leave a long-lasting imprint on morbidity and life expectancy in old age.

Eosinophil Effects in Mouse Models of Asthma

An assortment of leukocyte subsets are recruited to the lung during an asthmatic episode and accompany immediate changes to the mucosal lining, as well as long-term airway remodeling. Eosinophils are dominant among these infiltrating cells, but their presence has, so far, been linked only indirectly with disease (see the Perspective by **Wills-Karp and Karp**). **Lee *et al.*** (p. 1773) used a mouse model in which cell lineage–specific deletion of eosinophils could be achieved. In these animals, challenge with an allergen normally able to elicit a robust asthma-like response failed to generate significant pulmonary dysfunction or mucus accumulation. In a different eosinophil-deficient mouse line generated by **Humbles *et al.*** (p. 1776), these acute aspects were not significantly affected, but over the long term, these mice were protected from peribronchiolar collagen deposition and increases in airway smooth-muscle mass.

CREDIT: LEE *ET AL.*

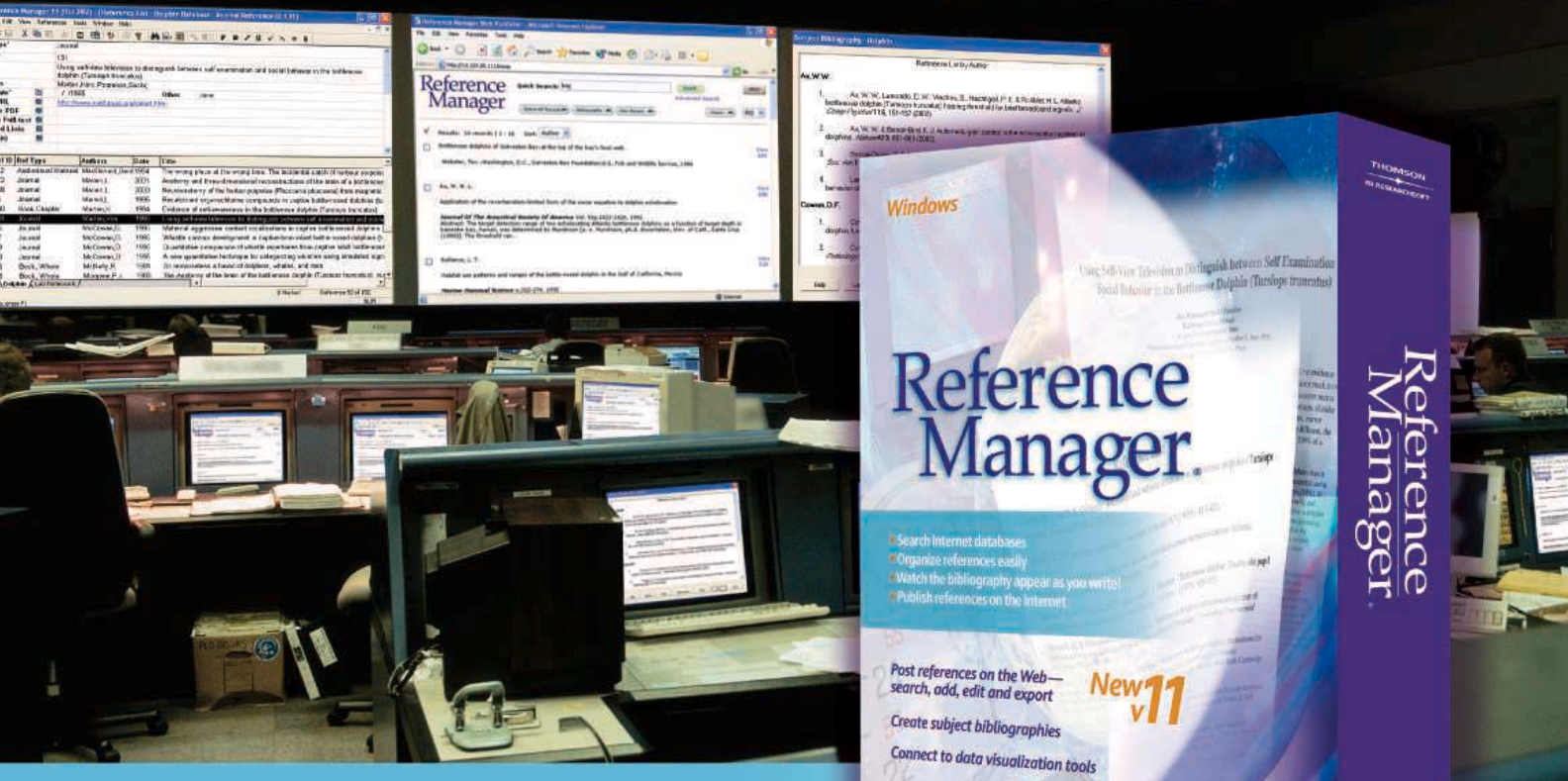
Dissecting the Evolution of a Sign Language

Human languages are digital in the sense that they are formed from discrete units. Is the brain predisposed toward dealing with sounds, words, and phrases, or are the existing languages that we learn simply structured discretely? **Senghas *et al.*** (p. 1779; see the Perspective by **Siegal**) offer evidence in support of the former view, drawing upon a population of deaf individuals in Nicaragua who have developed a new sign language. Descriptions of complex motion events are segmented into separate gestures representing the manner of movement (such as rolling) as well as path (such as downward).

How Sweet Is Your Tomato?

Quantitative traits suggest an underlying complexity of metabolism because gradations of a particular phenotypic trait make themselves apparent. The sweetness of tomatoes, particularly those tomatoes used for making ketchup, is one such trait. **Fridman *et al.*** (p. 1786) now analyze near-isogenic lines to identify the particular point mutation in an invertase enzyme that is responsible for gradations of sweetness in tomatoes. Unlike many other quantitative traits, which are often the summed result of several mutations, this sweetness gene acts on its own.

BIBLIOGRAPHY CENTRAL



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Talking with North Korea

The week-long visit of *Science*'s Richard Stone to North Korea (p. 1696) provides a fascinating new take on this strange land. He was shown allegedly cloned rabbits (interesting if true), just a few months after U.S. nuclear scientist Sig Hecker was handed a glass jar supposedly containing homemade plutonium (frightening if true). All this comes amid frustrating, sporadic six-party talks about North Korean nuclear and missile activities and the collapse of the Framework Agreement of 1994: the deal that supposedly froze their nuclear program in return for fuel oil and reactor construction. More ominously, it now appears that North Korea has a secret uranium enrichment program, and U.S. intelligence estimates that they may have recently reprocessed spent fuel into enough plutonium to make as many as six nuclear bombs.

North Korea has some 22 million people. About a quarter of these receive international food assistance, and refugees risk flight to an unwelcoming China. North Korea also maintains a million-man army, pursues major nuclear and missile programs, and threatens Seoul with entrenched conventional weapons. Yet this troublesome pariah nation reportedly has a scientific and technical community of 1.9 million people—poorly equipped but knowledgeable and congenial, Stone found, and eager to begin scientific exchanges with the United States and Europe. This would be a clear change in policy. During Secretary of State Madeleine Albright's visit to Korea in late 2000, the United States reportedly proposed exchanges (not necessarily scientific), but the idea was rejected by the Koreans.

There will be different U.S. reactions to this new prospect for engagement. Those who respond to countries that disagree with us by seeking to isolate them will call it a ploy to steal U.S. technology and will reject it outright. Another group will embrace it, hoping to begin constructive discussions with at least some people from this hyper-xenophobic country. A third group will want to use it as leverage to gain concessions; if those are not forthcoming, they will drop the idea. (Although scientific cooperation can often be a diplomatic sweetener, it rarely offers much leverage for securing major concessions.)

Everyone is a prisoner of his personal history. I went through the Cold War as an inveterate engager, as the first U.S. scientific attaché in Eastern Europe in the late 1960s, where I interacted with scientists that were more on our side than that of their own governments. Later I helped create the first U.S.-USSR Joint Committee on Science and Technology Cooperation, one element of the Nixon-Brezhnev detente agreed on at their 1972 summit meeting; and I was also involved in the first, mutually cautious science exchanges with the Chinese, ending 22-plus years of no contacts at all. Repressive governments characteristically try to prevent their people from having contacts with Americans, but those contacts are to our advantage because the contagion of freedom and democracy is dangerous for totalitarian societies, not the other way around.

Such an engagement strategy is what Joseph Nye, the dean of Harvard's Kennedy School, calls the use of "soft power." U.S. scientists took political risks in reaching out to Soviet physicist Andrei Sakharov and his colleagues in post-McCarthy America, and they generated enough mutual trust to influence the positions of both governments. That eventually led to a series of arms control agreements and helped both countries survive the U.S.-Soviet nuclear standoff in the era of mutual assured destruction. George Kennan, America's most prescient diplomat in the post–World War II period, created the Cold War containment strategy used against the USSR. But he argued for an engagement strategy with the Russian people and later lamented the heavy U.S. emphasis on containment in military terms and the relative neglect of available economic, political, psychological, and cultural tools.

These days, approaches employing soft power to build scientific and cultural bridges are often derided. But soft power may be even more important than before in a multipolar world in which terrorism and rogue states present different challenges to democratic institutions. Scientific and technical cooperation can be an effective instrument for wielding that power. So if the North Koreans are serious, if they want to begin modest scientific exchanges on peaceful uses of science, I would jump at the opportunity—in a cautious and constructive way. The world needs soft power, and more of it. In North Korea and elsewhere, these are the weapons that must ultimately prevail.

Norman P. Neureiter

Norman P. Neureiter is director of the AAAS Center for Science, Technology, and Security Policy in Washington, DC.

CREDIT: R. STONE/*SCIENCE*

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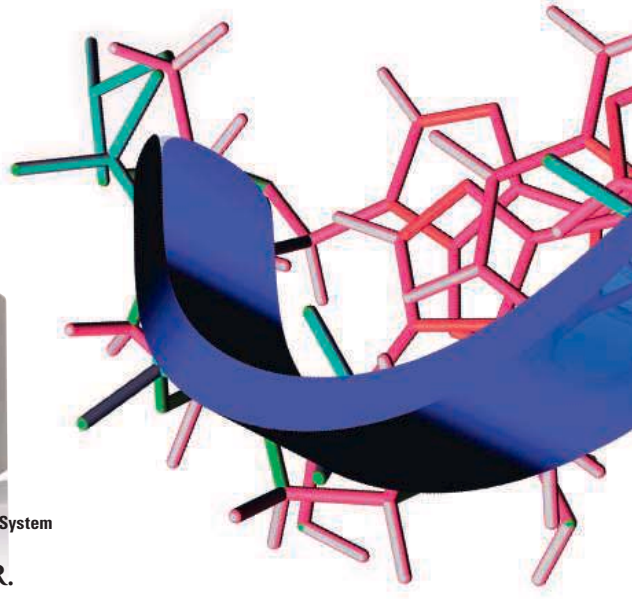

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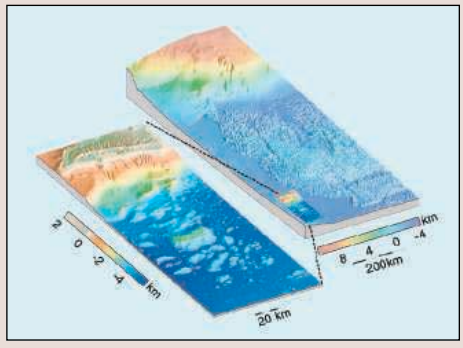
PLANETARY SCIENCE

Paradise Lost?

The dry and barren landscape on Mars is often compared to dry and desolate deserts on Earth, but McGovern *et al*. have chosen a tropical paradise, the Hawaiian islands, for a terrestrial analogy to explain the evolution of Olympus Mons, which is the largest known volcano (about 23 km in height and 600 km in diameter) in the solar system. It is partly bounded by an irregular scarp as high as 10 km, and lobes of hummocky terrain, which are called aureole deposits, funnel outward from this scarp. The aureole deposits contain remnants of formerly continuous volcanic flow units and morphologically resemble landslides around the edges of Hawaiian volcanoes. The authors suggest that, in similar fashion, Olympus Mons may have grown and spread by basal detachment faults. In Hawaii, the landslides are lubricated by high pore fluid pressure on the faults and are mostly submarine, which poses the question: Was Olympus Mons once a fluid paradise, too? — LR



Morphologies of the Nuuanu slide off Oahu (left) scaled in horizontal dimension to the aureole deposits of Olympus Mons (right).

J. Geophys. Res. **109**, 10.1029/2004JE002258 (2004).

ECOLOGY/EVOLUTION

Swifter, Higher, Stronger

Sexual selection, the evolutionary corollary of mate choice, is generally studied in organisms where direct matings (for example, internal fertilization) between individuals take place. The variance in male mating success that results when females choose, in particular, can lead to the evolution of showy and sometimes bizarre signals of male quality. However, the ancestral condition for sexual reproduction in animals is broadcast spawning and external fertilization—that is, the release of sperm and eggs by benthic marine organisms into the water column. Does sexual selection operate under these conditions?

Spawning sea urchin.

In an experimental study of reproduction in sea urchins, Levitan finds that sexual selection—as identified by the difference between males and females in the variance for fertilization success—does indeed occur, but only at intermediate population densities of males and females. At low and high densities, the variance in fertilization success did not differ between the sexes, because of sperm limitation at low density and sperm competition at high density. Hence, sexual selection in sea urchins is under control of the adult only in the sense of timing and quantity of gamete release; the rest is mediated by traits of the gametes themselves. — AMS

Am. Nat. **164**, 298 (2004).

MATERIALS SCIENCE

Fast-Flowing Filters

Porous membranes are used extensively for separation processes such as water purification. A current challenge is to fabricate membrane materials that can separate objects differing in size by only a few nanometers (which means small pores) and can still operate at a reasonable filtration rate (small pores are prone to blockage).

Akthakul *et al*. have enhanced the filtration capabilities of a commercial poly(vinylidene fluoride) (PVDF) membrane by spin coating a thin film of a copolymer consisting of a PVDF backbone, with short polyethylene oxide (PEO) side chains grafted on via a methacrylate linkage. The PEO and PVDF segments do not like to mix with each other, so the chains segregate locally into partially crystalline PVDF regions separated by PEO nanochannels. Water is repelled by the PVDF but is able to move through the PEO regions, thus enhancing the overall transport through the commercial PVDF membrane. The PEO segments interact strongly with the water molecules, which prevents organics from clinging and fouling the membrane. The membranes can also be used for molecular sieving, as demonstrated by the separation of similarly charged dye molecules, and for size-exclusion chromatography, as demonstrated by the separation of vitamins B2 and B12. — MSL

Macromolecules 10.1021/ma048837s (2004).

BIOCHEMISTRY

One Size Fits Many

Enzymatic reactions generally demand a precise positioning of catalytic residues; thus, structural disorder in a protein might be expected to be inconsistent with catalytic prowess. However, Vamcava *et al*. show that a monomeric chorismate mutase (mCM), obtained by redesign of the naturally occurring dimer, displays many of the characteristics of a molten globule yet still possesses one-third of the wild-type catalytic efficiency. Spectroscopic and thermal denaturation experiments all suggest that the monomeric form has high conformational flexibility and only adopts an ordered structure when a transition-state analog (inhibitor) is added. In contrast, dimeric CM is ordered both in the absence and presence of ligand. The polar character of the active site in the interior of mCM, unlike the hydrophobic core of the wild-type enzyme, fails to rigidify the folded state. When the inhibitor binds, it fills the pocket and supplies interactions that propagate and improve global ordering, as in the induced fit

CONTINUED ON PAGE 1681

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model of enzyme catalysis, in which the catalytically active conformation is locked into place as the reaction progresses. The idea that folding and catalysis can be linked implies that modern-day enzymes could have evolved from molten globules. Perhaps, a primordial structural plasticity conferred relaxed substrate specificity enabling a limited set of protein enzymes to catalyze a wide range of reactions. — VV

Proc. Natl. Acad. Sci. U.S.A. **101**, 12860 (2004).

MICROBIOLOGY

Thermophilic Parasite

Malaria is responsible for the death of more than 1 million people each year. In the course of cycling between the mosquito vector and the human host, the malarial parasite *Plasmodium falciparum* is exposed to high temperatures, up to 41°C in febrile patients, which are sufficient to send the microbe into heat shock.

Pavithra *et al*. examined the role of heat shock proteins in the development of the parasite within infected red blood cells by periodically incubating them at elevated temperatures, mimicking the recurrent febrile episodes typical of malarial infections. They find that elevated temperatures promote parasite development within the erythrocyte and that an inhibitor of one of the heat shock proteins actually disrupted parasite development. These findings support the idea that the parasite exploits the environmental cues provided by elevated body temperature to stage its development during infection, and it suggests that interventions that affect the malarial heat shock response may be useful in combating the disease. — SMH

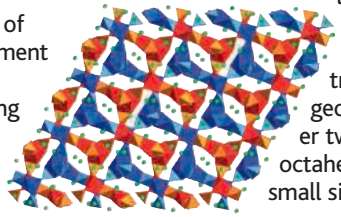
J. Biol. Chem. 10.1074/jbc.M409165200 (2004).

CHEMISTRY

Rare Frameworks

Many transition metals have been shown to form solid-state compounds with interpenetrating frameworks, which are of interest as they can provide routes to creating microporous materials. However, for the lanthanides and actinides, progress has been slower, with the only known example being an actinide compound, the thiophosphate UP_4S_{12}.

Aitken and Kanatzidis report that the reaction of ytterbium in a potassium thiophosphate flux yields $K_6Yb_3(PS_4)_5$. X-ray crystallography revealed two interlocked networks with three types of Yb^{3+} centers linking the PS_4 tetrahedra, one with the expected bicapped trigonal prismatic geometry and the other two with a distorted octahedral structure. The small size of Yb relative to other lanthanides appears to be the key factor in allowing it to adopt the octahedral geometry needed to form this type of network. — PDS



The interpenetrating lattices in red/orange and blue/light blue; K^+ in green.

CREDIT: AITKEN AND KANATZIDIS, *J. AM. CHEM. SOC.* 10.1021/JA0474648 (2004)

J. Am. Chem. Soc. 10.1021/ja0474648 (2004).

HIGHLIGHTED IN *SCIENCE*'S SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

Science's STKE www.stke.org

Moving TRPs to the Membrane

Singh *et al*. report that cation channels of the transient receptor potential (TRP) family are dynamically inserted into the plasma membrane in response to ligand stimulation of G protein–coupled receptors, as recently found after stimulation of receptor tyrosine kinases. The authors identified proteins involved in exocytosis—vesicle-associated membrane protein 2 (VAMP2) and α soluble *N*-ethylmaleimide–sensitive factor attachment protein (αSNAP) as interacting partners for the N-terminal domain of TRPC3 in a yeast two-hybrid screen. The interaction with proteins involved in exocytosis was confirmed with heterologously expressed proteins in transfected cells and endogenously expressed protein in rat brain. Exposure of human embryonic kidney cells expressing TRPC3 to the GPCR ligand carbachol resulted in increased abundance of TRPC3 at the cell surface, and this insertion was inhibited by cleavage of VAMP2 with tetanus toxin. Measurements of calcium influx with fluorescent indicators verified that the channels were functional. Thus, regulated insertion appears to contribute to agonist-stimulated TRP activity and calcium signaling. — NG

Mol. Cell **15**, 635 (2004).

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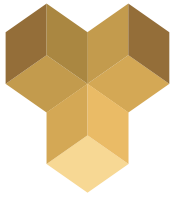

YOUNG SCIENTIST AWARD

NEW NAME NEW DEADLINE SAME GREAT PRIZE!

The Amersham Biosciences, now part of GE Healthcare, and *Science* Prize for Young Scientists has changed its name to the Young Scientist Award.

ILD IMMUNITY
HE WORLD

Immunological memory efficiently protects us from dying from infections caused by bacteria or viruses. However, some microbes change so fast that memory is never achieved and diseases caused by such agents are resistant to traditional vaccination, presenting a serious challenge for medical science worldwide. Dr. Marilia Cascalho is working on ways to overcome the limitations of vaccination, creating immunity even to viruses that can change.

Effective vaccination requires immune competency. Thus individuals that are immuno-deficient cannot effectively be vaccinated against infectious diseases. Dr. Cascalho together with her collaborators at the Mayo Clinic, Drs. Platt and Ogle, discovered a mechanism for rebuilding immunity in people with reduced T cell diversity, which will be valuable in treating patients with HIV and following transplantation or chemotherapy.

Dr. Cascalho became a regional winner of the 1999 Prize for Young Scientists with an essay on the discovery that DNA repair contributes to mutations in the immunoglobulin genes that are central to the development of immunological memory and effective vaccination. She believes the prize has played an important part in her subsequent progress. "And it shows that revolutionary contributions to science can be recognized even at an early stage in your career."

YOUR OPPORTUNITY TO WIN IS NOW

The Young Scientist Award was established in 1995 and is presented by *Science*/AAAS and GE Healthcare (formerly Amersham Biosciences). The aim of the prize has been to recognize outstanding Ph.D. graduate students from around the world and reward their research in the field of molecular biology.

This is your chance to gain international acclaim and recognition for yourself and your school. If you completed your Ph.D. in molecular biology* during 2003, describe your work in a 1,000-word essay. Then enter it for the **2004 Young Scientist Award**. Your essay will be reviewed by a panel of distinguished scientists, who'll select one grand prize winner and up to seven other winners. The grand prize winner will get his or her essay published in *Science*, receive US$25,000, and win a trip to the awards ceremony in Washington, D.C. The closing date for entries is **October 8, 2004**.

Go to **www.aaas.org/youngscientistaward** to find the entry form and award rules. We wish continued success to Dr. Cascalho. And to you.

Read Dr. Cascalho's latest findings in *J of Immunol.* 172:4709-4716 2004.

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* For the purpose of this prize, molecular biology is defined as "that part of biology which attempts to interpret biological events in terms of the physico-chemical properties of molecules in a cell" (*McGraw-Hill Dictionary of Scientific and Technical Terms*, 4th Edition).

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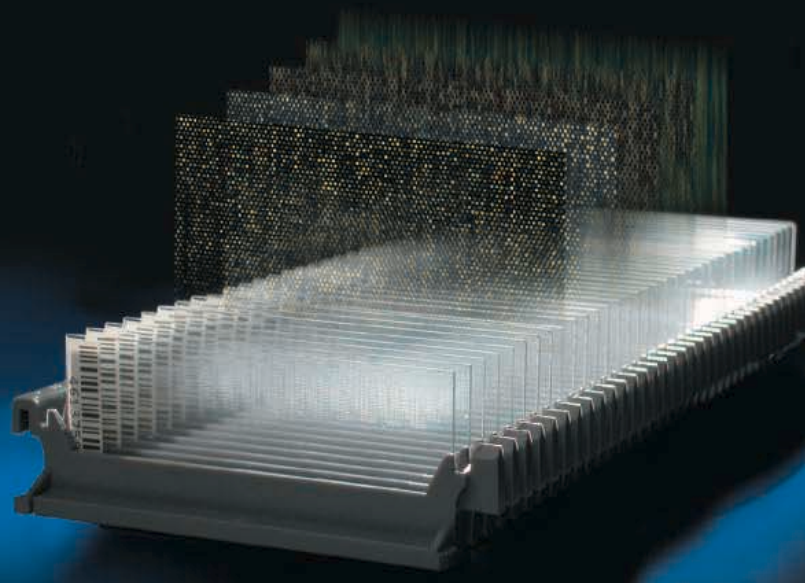

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NetWatch

edited by Mitch Leslie

EDUCATION

Limulus in the Limelight

The American horseshoe crab (*Limulus polyphemus*, below) is a laboratory star. Its blue blood clumps in response to certain microbes, inspiring today's standard test for identifying bacterial contamination. Studies of the crab's compound eyes led to Nobel Prize–winning research on the neurophysiology of vision. To learn more about these creatures, which are actually closer kin to spiders than to true crabs, visit these sites.

A basic primer from the University of Delaware* probes subjects such as the crab's evolution—the earliest fossil is about 500 million years old—and natural history. Every spring, for instance, droves of horseshoe crabs scuttle ashore along the Atlantic and Gulf coasts to mate and lay eggs. A similar site from the Delaware-based Ecological Research and Development Group† highlights details of the crab's anatomy and development. It also supplies a hefty bibliography of horseshoe crab literature and features a gallery of art and photos. Both sites discuss threats to the crabs (*Science*, 21 May, p. 1113), such as beachfront development.

* www.ocean.udel.edu/horseshoecrab
† www.horseshoecrab.org

DATABASE

Federal Science Register

Could methanol fuel cells power an artificial heart? How did dark lizards adapt to the bleached background at White Sands in New Mexico? These are just two of the studies the U.S. government underwrites. This site from the Department of Energy offers one-stop searching of federally funded research. You can prowl synopses of more than 500,000 current and recently completed projects sponsored by six agencies, including DOE, the National Science Foundation, the National Institutes of Health, and the Environmental Protection Agency.

www.osti.gov/fedrnd

IMAGES

Killers in the Forest

The fungus *Discula destructiva* besmirched this creamy dogwood bloom (right) and can eventually slay the tree. The parasite, which is devastating dogwoods in the East and West, is just one of the non-native organisms gnawing, sucking, and sliming their way through U.S. forests. The new Gallery of Pests from The Nature Conservancy (TNC) briefly describes more than 30 insects, fungi, and other troublemakers. Many accounts include photos of the organisms and the damage they inflict, along with maps that illustrate their spread. The gallery is the latest addition to TNC's invasive species site, which includes a host of resources aimed at land managers. To learn more about pesky invasive plants, for instance, consult Australian expert Rod Randall's Big Weed List.

tncweeds.ucdavis.edu/index.html

IMAGES

Not of This World

There may not be life elsewhere in the solar system, but there is geology, such as Mars's 24-kilometer-tall volcano Olympus Mons and our moon's South Pole–Aitken basin, a vast crater. Map-a-Planet from the U.S. Geological Survey lets you chart the surface features of seven solar system bodies, including Mars, Venus, and four of Jupiter's satellites. You can download maps based on a variety of measurements. For instance, Venus aficionados can choose among seven data sets, such as radar and microwave emissions, captured by the Magellan probe. The Galileo spacecraft snapped the pock-marked surface (above) of Jupiter's giant moon Ganymede, which is larger than Mercury.

pdsmaps.wr.usgs.gov

DATABASE

Parsing RNA

Researchers looking for broad patterns in the sequence or structure of RNA may want to check out Transterm, a tool from the University of Otago in New Zealand. The site lets users analyze RNAs from organisms whose gene sequences are housed in GenBank. For example, visitors can select a species and then determine how often its RNAs use each of the three-letter codons that designate a specific amino acid. The site also ferrets out motifs: nucleotide sequences or structural quirks that can affect the RNA's stability and how the cell reads it.

uther.otago.ac.nz/v5g.html

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

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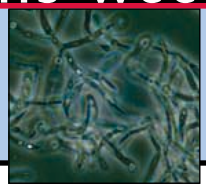

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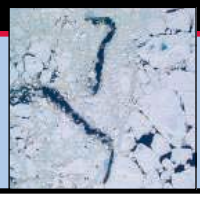
Don't lock up pathogen genomes



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When the Arctic was subtropical

MANAGING SCIENCE

House Votes to Kill Grants, Limit Travel to Meetings

For most scientists, having their research cited on the floor of the U.S. House of Representatives would be a crowning achievement. But for University of Missouri, Columbia, psychologist Laura King, it was part of a "really scary, bizarre day" that culminated in a vote to block her work and that of a second psychologist. It came minutes after the House imposed a cap on international travel to scientific meetings. While fiscal conservatives are touting the events of 9 September as a victory against government waste, scientific organizations are fuming about what they see as an unwarranted intrusion into the scientific process.

The setting for last week's legislative fireworks was the 2005 budget for the National Institutes of Health (NIH) and its parent body, the Department of Health and Human Services (HHS). Last year House Republicans narrowly missed pulling the plug on several NIH studies on sexual behavior on the grounds that the work was inappropriate and a waste of money. An amendment to block funds for the projects failed by just two votes. This year, however, an amendment by Representative Randy Neugebauer (R–TX) to bar HHS from using 2005 funds for two psychology grants passed on a voice vote. The immediate victims were King's work on college students' perceptions of themselves and a study by Samuel Gosling of the University of Texas, Austin, on how students' choice of dorm room décor can reflect their personality and mental health.

The vote would not impact funding for the two grants, which has already been disbursed. And the prohibition could be altered or dropped when the bill is reconciled with one passed by the Senate, which has not yet acted. But scientific groups are alarmed by the precedent. "There's no question that Congress has an oversight function here, but we don't think that extends to making decisions about individual grants," says David Moore, head of governmental relations for the Association of American Medical Colleges. NIH Director Elias Zerhouni says, "We need to do everything possible to preserve our historically successful system of independent peer review."

King's momentous day began with a phone call from the office of her congressman, Representative Kenny Hulshof (R–MO). Armed with a quick e-mail from King, Hulshof defended King's work and placed her entire CV in the official record. But his arguments, along with those defending Gosling, did not prevail. "It's very disheartening," King says. "Any grant in the social sciences or behavioral sciences could be attacked on this same basis." "I was dismayed," says Gosling, adding that, like King, he believes House members lack the knowledge to assess the grants.

Trivial pursuit? Psychologist Sam Gosling's work on how personality can shape work and living spaces took a hit in the House.

Neugebauer disagrees. "Taxpayer dollars should be focused on serious mental health issues like bipolar disorders and Alzheimer's," he told his colleagues. He derided Gosling's research as "interior decoration" and summed up King's work as asking students to define a "meaningful day," which he said "the federal government has no business paying someone" to study.

Although the legislation doesn't require King or Gosling to return any money, the two investigators may not be out of the woods. King is planning to apply for funds to renew her grant, and because her grant number is included in the amendment, she may need to submit a completely new proposal to continue her work. This summer Gosling received a 3-year, $200,000 grant from the National Science Foundation (NSF). A spokesperson for Neugebauer says the congressman is weighing whether to introduce a similar amendment when NSF's spending bill, now mired in committee, comes before the full House.

Scientific societies are urging the Senate to reject the Neugebauer amendment when the NIH bill comes before it. Federation of American Societies for Experimental Biology president Paul Kincade also hopes that a pending NIH plan to require grantees to provide a lay-language summary of the public-health importance of their grants will help prevent such attacks in the future. "It's important for scientists to explain what we do," he says.

The House floor vote also approved another amendment exerting control over NIH. The proposal, from Representative Scott Garrett (R–NJ), orders HHS to send no more than 50 staff members to any single international conference. Garrett objected to the $3.6 million spent on the 2002 international AIDS meeting in Barcelona, to which HHS sent 236 people. The money might have been better spent on buying drugs for AIDS patients, he says. HHS global health chief William Steiger has recently announced a similar goal of sending no more than roughly 50 staffers to international conferences (*Science*, 10 September, p. 1552).

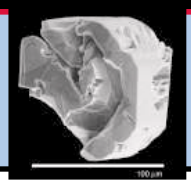
Strict enforcement of that limit—which genome institute director Francis Collins this week called "alarming"—could have a serious impact on several upcoming conferences, an NIH official notes, including a human genetics meeting in Toronto and two Keystone conferences on AIDS. The House and Senate could revise the wording to give HHS some wiggle room, for example, by exempting meetings in Canada. "It certainly is fixable," a staffer says. But in the meantime, for HHS scientists, foreign travel just got a little more complicated.

—JOCELYN KAISER

CREDIT: PHOTO BY JOHN LANGFORD

1696
North Korea quietly reaches out

1705
Dating the greatest mass extinction



1706
Biosecurity around the world

SPACE PROGRAM

Aiming for the Sun, Crashing to Earth

It was a gut-wrenching sight. As the capsule carrying precious samples of the solar wind collected by the Genesis spacecraft approached its Utah landing site, NASA TV viewers around the world could clearly see the 1.5-meter-wide, discus-shaped capsule tumbling earthward with no sign of its stabilizing parachute. Within seconds, the capsule slammed into the desert floor, abruptly ending the $264 million mission to return a sample of the sun for study of the solar system's origins.

All is not lost for Genesis, however. "There is still hope for science from this mission," says Genesis project manager Donald Sweetnam of the Jet Propulsion Laboratory (JPL) in Pasadena, California. The 205-kilogram capsule weathered its 360-kilometer-per-hour return surprisingly well, although it embedded itself halfway into the ground and cracked open. "We're quite confident we can achieve a high degree of success from a science point of view," says Genesis co-investigator Roger Wiens of Los Alamos National Laboratory in New Mexico. "Key collector materials have been determined to be very intact," says Donald Sevilla, Genesis recovery lead engineer at JPL. Brittle sample collectors did shatter, but pieces of collector may suffice for analysis.

With desert dirt driven inside the capsule and broken sample wafers falling out, "the major problem we have is contamination," says Sevilla. During its 3 years in space, Genesis had exposed various sorts of sample-collecting surfaces to the onrushing solar wind of atomic particles. Back on Earth, researchers planned to extract the embedded particles and determine their elemental and isotopic composition, which would precisely reflect the sun's present composition and thus the solar system's starting composition. That would help researchers understand everything from the formation of the solar system to the sun's acceleration of the solar wind. But the spacecraft's precious cargo is embedded only about 50 nanometers beneath the surface of the collectors. So specialists at NASA's Johnson Space Center in Houston, Texas, will have to not only put Humpty Dumpty back together again but also figure out how to clean collector surfaces without removing the samples.

And technicians won't be the only ones facing unexpected challenges. The disaster also aggravates NASA's struggles with its Discovery program of low-cost missions to the solar system (*Science*, 23 July, p. 467).

Down and dirty. Genesis PI Donald Burnett of Caltech sorts through some of the more heavily damaged solar-wind collectors (*inset*) following last week's crash landing of the sample-return capsule.

Discovery's CONTOUR spacecraft blew up in 2002 on its way to a comet, and several missions in the works or recently launched have encountered cost overruns and development problems.

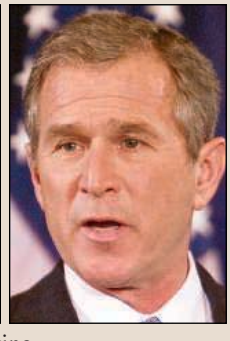
What, if anything, NASA can do to shore up management of ongoing missions will depend on the nature of the Genesis failure. Although the probe wasn't designed to send back data while entering the atmosphere, the recovery crew quickly determined that none of the explosives that deploy the parachutes had gone off, suggesting that the capsule's computer had never sent the command to fire. An onboard battery that had been acting up during the flight fell under immediate suspicion, but a mishap investigation board will take the next few months to determine a probable cause.

The Genesis disaster worries Peter Tsou of JPL, the deputy principal investigator of the Discovery program's Stardust mission, launched in 1999. Tsou notes that Stardust's sample-return capsule carrying comet dust was designed and built by the same industry partners as the Genesis capsule. "I'm keeping my fingers crossed" for the 2006 return, he says, but "frankly, there's not much we can do now."

–RICHARD A. KERR

SCIENCE POLICY

The Candidates Speak on Science



On 2 November, U.S. voters will decide whether to give Republican President George W. Bush a second term or put Democrat John Kerry in the White House. Continuing a presidential election-year tradition, *Science* has asked each candidate to lay out his views on more than a dozen science-related issues facing the nation. Their answers and an accompanying editorial are available online (www.sciencemag.org/sciext/candidates2004). The candidates' comments will also appear in the 1 October issue of the magazine.

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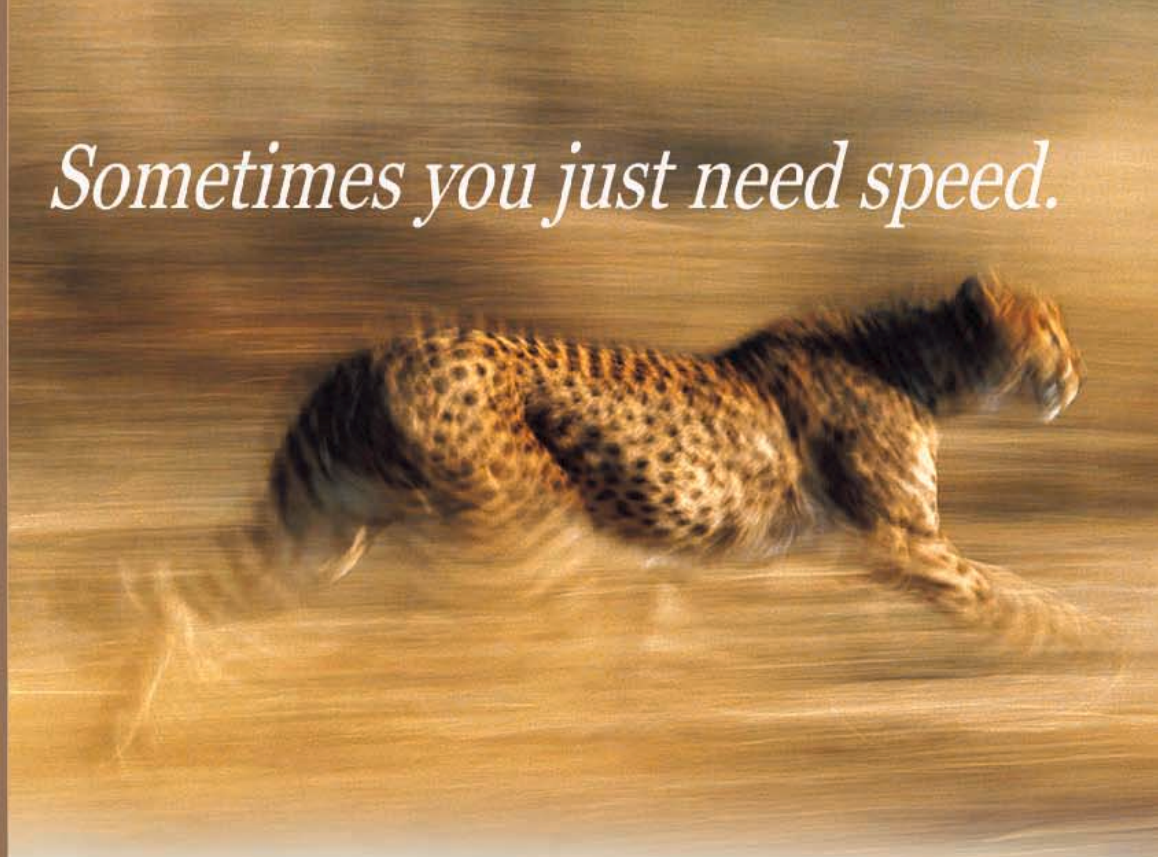

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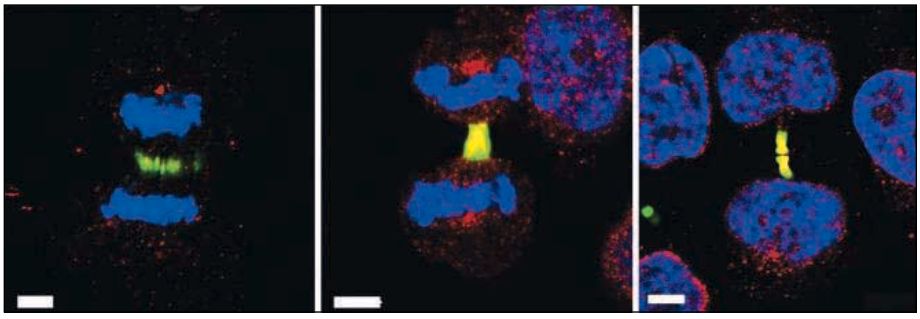
MEDICINE

Possible New Role for *BRCA2*

Cells with mutations in *BRCA2*, a breast cancer susceptibility gene, display a wide range of chromosomal abnormalities—everything from simple breaks to the gain or loss of whole chromosomes. Researchers think that this genomic instability, apparently the result of the inactivation of *BRCA2*, helps generate additional mutations that drive cells to become cancerous. New findings, described by Ashok Venkitaraman and his colleagues at the University of Cambridge, U.K., and published online this week by *Science* (www.sciencemag.org/cgi/content/abstract/1102574), now point to a novel way in which *BRCA2* inactivation may lead to cells that have abnormal chromosome numbers, a condition known as aneuploidy.

This work suggests that the loss of *BRCA2* function perturbs how dividing cells separate. If so, "that would be a new way to get [genomic] instability … and potentially interesting," says cancer gene expert Bert Vogelstein of Johns Hopkins University School of Medicine in Baltimore, Maryland.



CREDIT: M. J. DANIELS *ET AL./SCIENCE*

In the right place. As chromosomes (blue) separate during cytokinesis, *BRCA2* and proteins such as aurora-B kinase that are known to be involved in this last stage of mitosis colocalize (yellow).

Researchers, including Venkitaraman, had previously shown that the protein made by *BRCA2* is needed to repair chromosome defects, particularly the breaks in the DNA strands that can occur during DNA replication. Yet a mystery remained. Defective DNA repair resulting from *BRCA2* mutations "explains the abnormal chromosome structures [seen in cancer cells] but doesn't easily account for large changes in chromosome numbers," Venkitaraman says.

Work by others had hinted that *BRCA2* loss might also interfere with events during mitosis. To test this further, the Cambridge team determined how long it takes normal cells and cells in which either one or both copies of *BRCA2* was inactivated to progress from the onset of chromosome separation to complete daughter-cell separation. Cells with one inactive *BRCA2* copy took slightly longer than normal cells to separate. That interval was much longer—it was more than double the time for controls—for cells with two inactive copies. Indeed, many of those cells didn't separate at all and ended up with two nuclei.

Still, a holdup in cytokinesis could have simply been the indirect result of unrepaired DNA strand breaks. Dividing cells have ways to check for damaged chromosomes and can hold up mitosis until the damage is repaired. But the Venkitaraman team has other evidence that suggests to them that *BRCA2* plays a direct role in regulating cytokinesis.

In particular, they found that its protein localizes, along with proteins known to be involved in cytokinesis, in the central portion of the dividing cell and in the bridge that connects the daughters as they pull apart. "It's the first evidence to show that [BRCA2] is at the critical [cytokinesis] site rather than where we normally expect to see it in the nucleus," says breast cancer gene expert Simon Powell of Harvard's Massachusetts General Hospital. BRCA2 "not only has a role in repair, … but it has this additional role in cytokinesis," he concludes.

Cytokinesis experts aren't so sure, however. Alexey Khodjakov of the New York State Department of Health's Wadsworth Center in Albany says he is "not impressed" by the work, arguing that Venkitaraman's team has little evidence beyond the observed problems with cytokinesis in the mutant cells. "They don't have an explanation for how it happens," he says. Given that, there's still a possibility that the cytokinesis inhibition is the indirect result of impaired DNA repair in the mutants.

Venkitaraman concedes that that is still a possibility, but he says he and his colleagues are currently working to define *BRCA2*'s mechanism of action and hope to resolve the issue soon. **–JEAN MARX**

ScienceScope

NCI Backs Nano in Cancer War

National Cancer Institute (NCI) officials this week announced plans to spend $144 million over 5 years on nanotechnology efforts to fight cancer (*Science*, 23 July, p. 461). About $90 million will be used to establish at least five new multi-university centers of excellence over the next year aimed at using nanosized particles to create novel diagnostic, therapeutic, and imaging techniques. Another $38 million will flow to individual investigators and $16 million to training awards.

NCI has supported nano projects for the last 6 years, and most of the initiative's funds will come from repackaging existing efforts and terminating current programs, says NCI deputy director Anna Barker. Still, the time is right for such an effort, says chemist Richard Smalley of Rice University in Houston, Texas. Nanotechnology gives researchers a bevy of new approaches to targeting specific cells within the body, he says: "There is a brave new world out there for diagnosis and treatment." **–ROBERT F. SERVICE**

Panel Recommends Keeping German Cloning Ban, for Now

Germany's federal Bioethics Council has recommended that the nation maintain its moratorium on all forms of cloning—for now. But although the 25-member council last week unanimously called for a worldwide ban on reproductive cloning, its members split on the question of allowing research cloning, which uses nuclear transfer to develop stem cells from human embryos.

In the council's 13 September statement, one group of five members rejected all cloning research, calling it morally unjustified. A second group of 12 members said that research cloning should be allowed under strict rules. Five members said that research cloning should be prohibited for now but could be justified in the future if advances make it more likely to produce treatments. Despite the apparent majority for allowing cloning research (*Science*, 20 August, p. 1091), the panel urged the government to maintain its current moratorium.

Panel member and Nobel laureate Christiane Nüsslein-Volhard of the Max Planck Institute for Developmental Biology in Tübingen says she is pleased with the compromise. Although she supports regulated cloning research, she says current techniques are so inefficient in animal experiments that "it is premature" to move to human cells. Science minister Edelgard Bulmahn praised the report, saying she sees no reason to change Germany's embryo-protection law. **–GRETCHEN VOGEL**

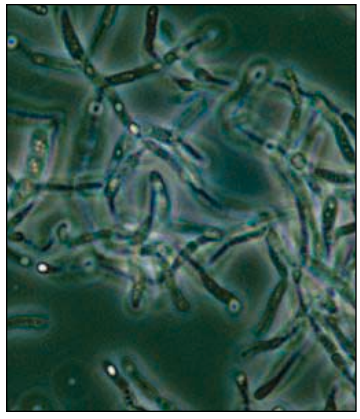
DATA SECURITY

Report Upholds Public Access to Genetic Codes

The possibility of bioterrorism shouldn't stop scientists from freely sharing genome data, concludes a new report from the National Academies' National Research Council (NRC). The study, requested by the CIA and the National Science Foundation, says that limiting public access to genome data on potential bioweapons is impractical and would do more scientific harm than good.

The U.S. government typically requires all federally funded scientists to make their genome data public. Since scientists sequenced the first viral genome in 1975, they have released the genetic codes of more than 1100 viruses and 150 bacteria, including those of the dangerous pathogens that cause smallpox, anthrax, and the plague. In the wake of the October 2001 U.S. anthrax attacks, however, some analysts have proposed restricting access to such data to make sure it doesn't fall into the wrong hands. They worried that would-be bioterrorists might draw upon the growing mountain of gene sequence data in public databases to engineer new bioweapons, such as unusually infectious viruses or toxic bacteria that resist drugs.

But "open access is essential if we are to maintain the progress needed to stay ahead of those who would attempt to cause harm," says Stanley Falkow, a microbiologist at Stanford University in Palo Alto, California, who led the new study (www.nap.edu/catalog/11087.html). It is unlikely that raw sequence data would help bioterrorists develop superweapons, the NRC panel says, and locking away information would harm efforts to improve biodefenses and fight emerging diseases such as severe acute respiratory syndrome. Coming up with workable restrictions would be difficult, the panel adds. The genomes of many dangerous pathogens are already in the public domain, and there is little agreement on what kinds of information should be put off-limits. If the government needs to keep genomic secrets, it says, it should use its long-standing authority to classify information.



Force for good. Academy panel wants genomes of potential bioweapons such as anthrax to remain public.

The panel's approach sits well with several scientists concerned about biosecurity. "This is the right decision, from the standpoints of both public health and security," says Barbara Hatch Rosenberg, a bioweapons expert at the State University of New York's Purchase College. "Stringent restriction would pose unacceptable costs," agrees molecular biologist Richard Ebright of Rutgers University in New Brunswick, New Jersey. "There are no 'biohackers' using genome data in basements." –DAVID MALAKOFF

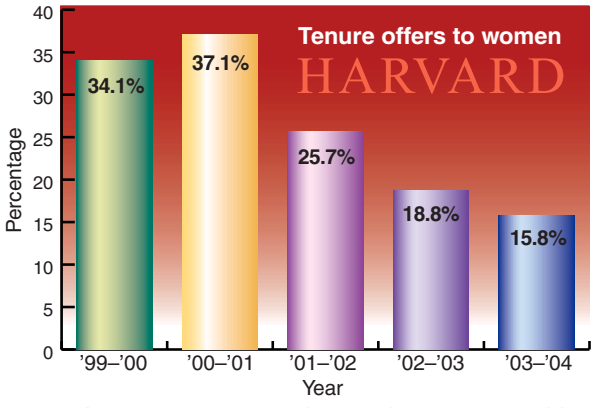
WOMEN IN SCIENCE

Harvard Faculty Decry Widening Gender Gap

The percentage of women offered tenured slots in Harvard University's Faculty of Arts and Sciences (FAS) has shrunk by half in the past 5 years. In a letter sent this summer to President Lawrence Summers and obtained by *Science*, some two dozen women faculty members called the dramatic drop an unintended result of policies put in place since Summers took office in 2001. Summers, in turn, blames departmental search committees for not looking harder for strong women candidates. Both sides agree, however, that the issue is worth talking about and have scheduled a sit-down next month to figure out how Harvard can do better.

"The whole concern about increasing diversity on campus has been downgraded," says a senior faculty member who, like other signers who spoke to *Science*, requested anonymity. "We'd hate to go back to a 1980s world at Harvard in which only 7% of tenured FAS faculty are women."

Women are generally underrepresented among the faculty of major research universities, and the situation becomes more pronounced as they ascend the professorial ranks. In theory, Harvard is in a better position to correct a gender imbalance than most universities, because it rarely awards tenure to those already on campus. But the share of women offered those coveted slots has slumped from 37% of the total pool in 2000–01 to 16% in the academic year that just ended (see graph). That's below the overall faculty ratio of 19.6%, posing a threat to hard-won gains during the 1990s.



Wrong direction. Women faculty members say Harvard has taken a step back in providing opportunities for women.

On 18 June, 26 tenured faculty women laid out their concerns in a three-page letter to Summers and FAS Dean William Kirby. They cited several possible contributing factors, including the elimination of an affirmative action dean in 2001 and the university's emphasis on hiring "rising young stars," an age cohort that one of the signers says "corresponds to a woman's child-bearing years."

On 23 July, Summers and Kirby wrote back. The quest for younger faculty, said Summers, should actually narrow the gender gap, because "the pool of women available in most fields is larger in cohorts at an earlier career stage." Kirby explained that affirmative action is a priority for four new division deans—positions created since Summers arrived—and added that new hiring policies will ensure more "broad and thorough" searches.

Summers and the petitioners concur that the key to improving the situation lies with how department chairs choose to fill their tenured slots. But the signers say Summers needs to lean more heavily on those chairs. "Most members of the search committee are men," says one petitioner, "and they'll often bring in a token woman candidate after they've decided to hire somebody else."

The two sides will discuss the matter at a lunch on 6 October. "We're hopeful about change," says a signer, "because Larry is smart and very educable." –YUDHIJIT BHATTACHARJEE

CREDITS (TOP TO BOTTOM): CDC; SOURCE: HARVARD UNIVERSITY

PALEOCEANOGRAPHY

Signs of a Warm, Ice-Free Arctic

Drillers returned to Tromsø, Norway, this week with sediment cores from the first holes ever drilled into the deep, ice-covered Arctic Ocean. The cores contain evidence of a dramatic defrosting of the Arctic Ocean near the North Pole 55 million years ago and a long, slow slide toward the perennial ice cover of recent times. Somewhere in the hundreds of meters of mud cored should be a record of the last ice-free Arctic summers of millions of years ago, conditions that may return in the greenhouse world of 2100.

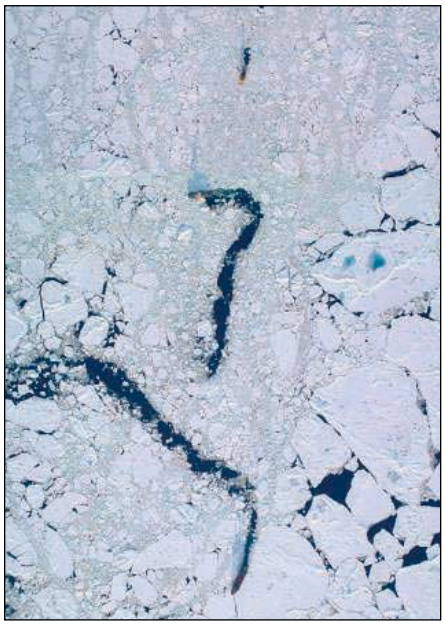
The deep-drilling success of the $12.5 million Arctic Coring Expedition (ACEX) comes after decades of merely picking at the upper few meters of Arctic seafloor sediments. Since the 1960s, scientific ocean drilling in other seas has returned 160 kilometers of rock and sediment cores. But scientific drill ships had to flee at the sight of ice, and in the Arctic only the top few meters of sediment could be sampled through the oceanwide ice.

Now, under the new Integrated Ocean Drilling Program (*Science*, 18 April 2003, p. 410), the 13-member European Consortium for Ocean Research Drilling has fielded a three-ship flotilla: an ice-reinforced drill ship to float 1300 meters above the drill hole plus two icebreakers—one of them nuclear-powered. At the drill sites, just 220 kilometers from the North Pole, ice as much as 4 meters thick covered the surface, usually with only a few small gaps. Despite a string of mechanical breakdowns—a crucial high-pressure pump valve broke three times—the ships were equal to the task. "We found that even in heavy ice conditions, we could stay [over the same hole] as long as 8 days," says Kate Moran of the University of Rhode Island, Narragansett, who with Jan Backman of Stockholm University in Sweden was an ACEX co-chief scientist. "We can probably go any place in the Arctic Ocean and drill."

In 3 weeks of drilling operations, ACEX bored through all 410 meters of sediment at one site on the underwater Lomonosov Ridge and drilled to shallower depths in five other holes. All told, the 19 shipboard scientists from eight nations gathered a total of 339 meters of sediment as old as 80 million years.

Their biggest find was a couple of hundred thousand years' worth of sediment from 55 million years ago. It contains animal and plant microfossils typical of 20°C subtropical waters, not the subzero waters of today. The fossils mark the so-called Paleocene-Eocene Thermal Maximum (PETM) recorded around the globe in marine sediments of the time. "Getting the PETM was a fabulous result," says Moran. Seismic probing of the site had suggested that sediments of PETM age were missing there.

Now, paleoceanographers can try to sort out the Arctic Ocean's role in the PETM. The warming seems to have been triggered by a massive release of methane, a greenhouse gas, stored beneath the sea floor as an icy hydrate (*Science*, 28 January 2000, p. 576). It's unclear what drove the methane release, but a geochemical peculiarity of the ancient Arctic might have been involved. ACEX scientists found strikingly low Arctic seawater salinities during most of the past 60 million years, due partly to large influxes of river water. Such low-density waters might have altered ocean circulation globally if they leaked into the Atlantic, Moran notes.



Ice eaters. Icebreakers (bottom and middle) run interference for the stationary drill ship (top).

CREDIT: CANATEC ASSOCIATES INTERNATIONAL, CALGARY

Once global Eocene warmth began to wane, the world was on its way toward the deep chill of the past few million years. The first sure signs of Arctic ice—bits of sand that must have rafted to mid-ocean in one-time grounded ice—appeared in 40-million-year-old sediments. That's earlier than some scientists had expected. Seven million years ago, the delivery of ice-borne sand picked up sharply, suggesting more and possibly year-round ice. But pinning down when Arctic summers last saw ice-free waters—a condition global warming might bring on by the end of the century—will require close inspection of the cores on shore, says Moran. She can hardly wait.

–RICHARD A. KERR

ScienceScope

Japan Revises Mad Cow Plans

Japan is scaling back its policy of testing all slaughtered cows for "mad cow disease" (bovine spongiform encephalopathy, BSE). But its new plan to test only slaughtered cows older than 20 months will still be the world's most stringent BSE screening program.

The new policy, set to begin later this month, was a compromise, says Takashi Onodera, a molecular biologist at the University of Tokyo and a member of a government advisory group. Europe and the United States test cows that are 30 months and older, he notes, in part because scientists believe younger cows haven't accumulated enough BSE-causing prions to be picked up by current tests. Japan's Finance Ministry also wanted to cut back on "useless testing" to trim the $30 million to $40 million annual cost, but the Ministry of Health and consumer groups were reluctant to raise the cutoff age any higher because Japan has found the disease in 21- and 23-month-old cows.

–DENNIS NORMILE

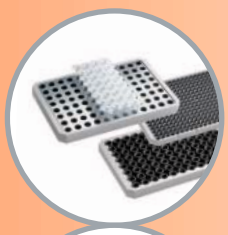
FDA Panel Approves ADHD Study

A controversial study that would expose healthy children to a stimulant should proceed, a Food and Drug Administration (FDA) ethics panel recommended last week. The pediatric ethics subcommittee decided that the study, on attention deficit hyperactivity disorder (ADHD), is ethically acceptable. But it urged sponsors to offer less compensation to enrolled families, saying a proposed $570 payment might unduly influence parents who needed the money.

The ADHD study is funded by the National Institutes of Health and led by child psychiatrist Judith Rapoport. It raised red flags among reviewers because scientists wanted to enroll both healthy children and those with ADHD, all aged 9 to 18 (*Science*, 20 August, p. 1088). All subjects would receive one dose of dextroamphetamine, a drug used to treat ADHD, and then undergo a magnetic resonance imaging scan to see whether the brains of healthy and ADHD children respond differently to the drug.

The subpanel's recommendation now goes to the full pediatric advisory committee, which will then make a formal recommendation to FDA.

–JENNIFER COUZIN



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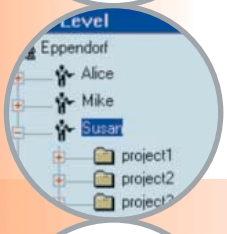

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DRUG RESEARCH

Legislators Propose a Registry to Track Clinical Trials From Start to Finish

Data from company-sponsored clinical trials are often treated as business secrets, but that practice may soon change. In the wake of allegations that a few companies have suppressed negative results to promote their drugs, some members of Congress say they intend to make it easier for the public to track clinical studies. Democrats plan to introduce legislation in both the House and Senate this month to create a mandatory public registry. It would require that all clinical studies be described publicly at their inception and that results be added when a trial is complete.

The proposal is part of a surge in efforts to overhaul the rules of clinical reporting. Last week, an international consortium of 13 medical journals announced that it would publish results only from clinical trials that were publicly registered when the trial began. Hurrying to head off legislation, the Pharmaceutical Research and Manufacturers of America (PhRMA), an industry trade group, reported that it will start a voluntary registry next month. Even pharmaceutical executives acknowledge a problem: "There is clearly a societal crisis in terms of credibility of drug company results," said John Hayes of Eli Lilly and Co. in Indianapolis, Indiana, at a hearing in the House last week.

More data. Concerned about access to drug test data, Representatives Edward Markey (*left*) and Henry Waxman are drafting a bill to require registration of all clinical trials from their inception.

One source of trouble, advocates of a registry say, is that clinical research suffers from "publication bias," a tendency to trumpet good results and bury the bad. A dramatic example came to the fore last year in a controversy over the safety and effectiveness of antidepressant drugs in children (*Science*, 23 July, p. 468). When the U.S. Food and Drug Administration's (FDA's) review of the antidepressant Paxil found that children taking it had higher-than-expected rates of self-harm, Paxil's maker, GlaxoSmithKline, released a batch of unpublished studies. The newly released data suggested that Paxil was ineffective in treating their depression. Glaxo's published study on Paxil in depressed youngsters had suggested that it worked. FDA later admitted that only three of the 15 pediatric antidepressant trials submitted to it by various companies had found the medications effective.

At a hearing last week in the House Energy and Commerce Subcommittee on Oversight and Investigations, FDA official Janet Woodcock came under sustained fire for the agency's reluctance to release negative data submitted by companies. Legislators asked Woodcock whether FDA had a responsibility to the medical community to publicize negative results. She said, "This is a conundrum for the agency," which must normally protect proprietary information.

Observers trace the current furor to the 1997 FDA Modernization Act, which offers drug companies a 6-month patent extension as a reward for testing drugs in youngsters. The legislation was prompted by the fact that many drugs approved for adults are often prescribed to children "off-label." The law didn't ensure that these study results would be released, however. A 2002 law sought to remedy the data gap by requiring FDA to post online summaries of results of all pediatric trials submitted to it for extra patent protection.

But the problem is bigger than pediatric testing, Representative Henry Waxman (D–CA) said at last week's hearing. "The pharmaceutical industry has systematically misled physicians and patients by suppressing information on their drugs," he said. Waxman and Representative Edward Markey (D–MA) are crafting a mandatory registry bill in the House, while senators Christopher Dodd (D–CT), Edward Kennedy (D–MA), and two others are writing a companion bill in the Senate.

The Waxman-Markey bill will hew closely to recommendations made by journal editors and the American Medical Association, according to a statement released by Waxman's and Markey's offices. It will demand disclosure of a trial's objectives, timeline, eligibility criteria, and funding sources. It also will require that results be promptly released. Because the bill's authors are concerned that earlier attempts to create a public registry were not backed by enough muscle, a congressional aide says, this version will be stringently enforced. For example, violators may be subject to fines.

U.S. Clinical Registry Proposal May Require:

- Registering all U.S. drug trials at their launch
- Listing eligibility requirements for participants
- Listing funding sources
- Posting results, including those not published in journals
- Fining noncompliant trial sponsors

Pharmaceutical companies, meanwhile, are racing to set up their own registries or pledging to participate in PhRMA's. "If we are running a trial, the public will know it," says Lawrence Olanoff, executive vice president of Forest Laboratories in New York City. Forest Labs, which makes the antidepressant drugs Lexapro and Celexa, last week announced that it would set up a trial registry as part of an agreement to end an investigation by New York Attorney General Eliot Spitzer. Other companies, including Eli Lilly and Pfizer, have pledged to release trial information and results, although the details differ.

Still, some say voluntary registries may prove disappointing, especially given that past attempts of this sort have faltered. Legislation "is the only route" to guarantee that a registry works, says Kay Dickersin, a clinical trials expert at Brown University in Providence, Rhode Island. Even a mandatory registry, though, isn't without potential pitfalls: Although she worries about "losing the moment," Dickersin concedes that some trial results could be confusing and will require careful handling when posted.

–JENNIFER COUZIN

At once coy and eager, North Korea's scientists are striving to forge new alliances with Western researchers without abandoning their unique philosophy of steely self-reliance

A Wary Pas de Deux

PYONGYANG—Ri Hak Chol leads the way out of the main building of the Institute of Experimental Biology, under a façade declaring that "only our Great General lives, the rest of us fight." We walk past a volleyball court—the game is a popular lunchtime activity at the institute—and enter a dim room filled with wooden cages and the pungent smell of animals. Under a window letting in weak light on an overcast day, one of the most remarkable achievements of North Korea's scientific community sits passively behind bars: a white rabbit. It's one of a half-dozen that Ri and his colleagues claim to have produced since 2002 through somatic cell cloning, the technique that gave birth to the famed Scottish sheep Dolly in 1997.

Western scientists may soon get the chance to scrutinize the cloning claim, published only in North Korea, and take the measure of the country's finest young scientists, including the 37-year-old Ri. In a momentous shift in policy, the government of the Democratic People's Republic of Korea (DPRK) this year has given a green light to select scientists to team up with Western colleagues on joint research projects.

Some veteran watchers of the Hermit Kingdom say that its version of glasnost offers historic opportunities. Any light shed on the country's largely enigmatic scientific community will help Western experts gauge its capabilities. Moreover, the possibility now exists for an innovative brand of diplomacy to proceed in parallel with traditional channels. "Scientific diplomacy can help North Korean intellectuals to survive and can inject, very gradually and cautiously, modern values into North Korea's still very isolated society," argues Vasily Mikheev, chair of the Asia security program of the Carnegie Endowment for International Peace in Moscow.

Exotic land. A tour guide wearing a traditional *hanbok* at Tongmyong temple.

Visiting the Hermit Kingdom

North Korea rarely grants visas to foreign journalists, and those it does invite are often steered to tourist zones or choreographed events such as festivals. For the most part U.S. journalists are persona non grata. But early this year the North Korean government signaled a willingness to allow some scientists to interact with Western peers. In June, a few days after North Korea and the United States exchanged substantive proposals in nuclear talks in Beijing, the Academy of Sciences of the Democratic People's Republic of Korea invited me to visit some of its premier labs. As part of the deal, I also got a fascinating glimpse of life in this reclusive country.

On my first morning in Pyongyang I was awakened at 5 a.m. by a melancholy sound blaring from loudspeakers on the street. The melody, more than 3 minutes long, is titled "Where Are You Now, Our Great General?"—a hymn to Kim Jong Il, the current leader. His deceased father, Kim Il Sung, is the Great Leader. Except when preempted by occasional tests of the city's air-raid sirens, the tune is played every hour on the hour from early morning to late evening. After a hotel breakfast of rice, fish, and kimchi (spicy pickled cabbage), my escorts, one a conservation biologist and the other an academy officer, met me in the lobby. As we waited for the van to arrive, I noticed that the biologist was wearing a different loyalty badge from the previous day. All adults wear them, small pins with the face of Kim Il Sung often set against a red background. "What happens if you forget to wear it?" I asked her. "Nothing, it's not a problem," she insisted before adding earnestly, "but we would *never* forget our Great Leader."

As we drove across town later that day we passed the Grand People's Study House, the city's central library built in a majestic pagoda style—a refreshing departure from the general vista of bland concrete office buildings and apartment towers. In lieu of traffic lights, shut off to conserve electricity, traffic wardens—attractive young women wearing white caps, smart white jackets cinched with brown belts with big silver buckles, royal blue skirts, and short white boots—stand ramrod straight in the middle of major intersections, their eyes darting in each direction before using batons to signal to drivers. Cars need a special permit to operate on Sundays, so on that day the streets are nearly empty—and the traffic women get a day off.

The few dozen scientists I met in North Korea struck me as warm, open-minded, and eager to cooperate with Western colleagues. Such collaborations will rely on the government's good graces, of course. Nothing can be taken for granted: Government operations are more opaque than ever, with Party conferences and other meetings once reported in the state newspaper now held behind closed doors. "We have no idea how decisions are made," a Swedish diplomat told me. Yet my visit left me feeling that scientific exchanges are inevitable—and will benefit Koreans and Westerners alike. **–RICHARD STONE**

Lapin-ectomy. A pair of young researchers performs embryo transfer on a rabbit in a clean room at the Institute of Experimental Biology.

CREDITS: R. STONE/*SCIENCE*

Leading the charge. A 10-meter-long mural at the Academy of Sciences' Unjong regional branch depicts Kim Jong Il "giving guidance" to researchers.

Science policy officials attuned to the promising signals from Pyongyang are scrambling to seize the initiative. Next month, for example, a three-member delegation from the Academy of Sciences of the DPRK (ASK), with sponsorship from the Ford Foundation in New York City, is planning to visit London to discuss with officials and granting bodies the ground rules for potential collaborations. And a major symposium on scientific cooperation with North Korea, to be held in Moscow, is in the works for April 2005.

Any joint project would entail some risk. There's the matter of *Juche*, for starters. The word stands for an all-encompassing credo conceived by longtime leader Kim Il Sung and described as a "man-centered philosophy" grounded in steely self-reliance. Some experts warn that *Juche* could turn collaborations into a one-way vacuuming of information and expertise by a regime that has recently set the world on edge over its attempts to develop what it calls a "nuclear deterrent" (see p. 1698). Would-be partners must vet projects for potential usefulness to North Korea's military, with the assumption that any equipment and materials provided under a grant could be diverted for weapons R&D. "If there's even the slightest possible military application, they will use it," warns a Swedish diplomat who has lived in Pyongyang. "That makes everyone very nervous."

North Korean officials have reasons of their own to be anxious. Interactions between their intellectual elites and Western scientists inevitably would raise awareness of modern Western life. With the Internet off-limits to most North Koreans, the government has imported scientific information primarily through its diplomatic posts and distributed it by means of a countrywide "intranet" (see p. 1701). A freer diffusion of knowledge could undercut the chillingly effective cloak of naiveté about the outside world that the North Korean government has draped over its citizenry. "It could put a strain on the system," says the Swedish diplomat.

Dead ringer? One of the claimed clones.

In an unprecedented trip by a Western journalist, *Science* in July toured a handful of ASK's premier biotech and computer science laboratories and its science university. The picture that emerged is one of dedicated scientists toiling in largely antiquated and poorly supplied facilities—and hungry for contact with the outside world.

Self-reliant scientists

After rising from obscurity to lead North Korea in 1945, Kim Il Sung, a former guerrilla fighter against Japanese colonial rule, rapidly consolidated power and ruled the North until his death in 1994. Kim remains the country's "president for eternity," chemically preserved and lying in rest in the lavish Kumsusan Memorial Palace, his former state residence. After his death, the palace was converted to a "supreme temple of *Juche*," displaying mementoes of his rule, including a railway carriage he used and honorary awards such as doctorates and a pin from an Italian lawyers' association. Hewing to a *songun*, or "army-first," policy, North Korea is perhaps the most heavily militarized nation in the world, with a standing army of approximately 1 million soldiers and enough artillery trained on Seoul—just 40 kilometers south of the Demilitarized Zone, or DMZ, that separates the two countries—to obliterate the South Korean capital if war were to break out.

Just below the military on the state's pedestal of honor is the scientific community. One of its heroes from North Korea's early days is Li Sung Ki, a chemist who with two Japanese colleagues during World War II invented a polymer, vinalon, still used in the production of everything from clothing to fishing nets. During the 2 decades after the Korean War (called the "Fatherland Liberation War" here) ended in 1953, the economy of the heavily industrialized North was booming. Seduced by the apparent economic miracle, Sweden got an early foothold in North Korea, establishing an embassy in Pyongyang in 1975. It began

CREDITS: R. STONE/*SCIENCE*

Nukes for Windmills: Quixotic or Serious Proposition?

PYONGYANG—The Democratic People's Republic of Korea (DPRK) often resorts to fiery rhetoric when faced with challenges from the outside, but it knows about conciliatory tactics as well. Indeed, an unofficial envoy who spoke with *Science* here in July claimed that the government is ready to make a remarkable concession: It would abandon its nuclear program in its entirety—both weapons and power generation—in exchange for "clean energy technologies" such as wind power. The proposal, if backed by the government, could set the stage for progress in high-level discussions over the fate of North Korea's nuclear program. But like many other signals from Pyongyang, this one is hard to interpret.

"It's the first time we've heard of [trading nuclear power for clean energy technologies]. It's very intriguing," says Mikio Mori, who until last month was director for multilateral nuclear cooperation in Japan's Ministry of Foreign Affairs. That fits with what Kim Jong Il, leader of North Korea, told Japan's prime minister, Junichiro Koizumi, in talks in Pyongyang last June. "He tried to persuade Koizumi that having nuclear weapons is not in [North Korea's] benefit and that dismantlement is their final objective," Mori says. A request for clean energy technologies would be "a big plus, very positive," adds Robert Alvarez, a former senior policy adviser to the U.S. Department of Energy (DOE) who helped implement the Agreed Framework, a 1994 accord in which North Korea agreed to mothball its plutonium facilities for energy aid and other incentives. Alvarez is now at the Institute for Policy Studies in Washington, D.C.

Representatives of six countries—China, Japan, North Korea, Russia, South Korea, and the United States—have met intermittently since August 2003 to try to solve a crisis stemming from North Korea's efforts to acquire what it calls a "nuclear deterrent." At the most recent round of talks last June, North Korea proposed what it terms a "reward for freeze": energy aid in exchange for a promise to suspend its plutonium program once again. The United States, in contrast, offered that non-U.S. parties would provide heavy fuel oil only after North Korea agrees to dismantle all its nuclear programs. "There are major gaps between the North Korea and U.S. proposals," says Mori. "We need to define clearly what the parties want." The next round of talks had been expected to take place in Beijing later this month. However, revelations about uranium-enrichment experiments in South Korea and a recent flare-up in the war of words between North Korea and the United States threatened a delay until after the U.S. presidential elections.

The true nature of North Korea's "deterrent" is far from clear. The country's ambitions to build a nuclear weapon first came to light in the late 1980s. At a meeting of deputy foreign ministers of Soviet bloc states in Pyongyang in 1988, North Korean officials leaked to the Soviet Embassy that their military had started a nuclear weapons program, says Vasily Mikheev, Asia expert for the Carnegie Endowment for International Peace in Moscow. After an investigation, the KGB concluded that it was unlikely—but not impossible—that North Korea could devise a working bomb, Mikheev says. Russia's opinion remains unchanged, he says: "North Korea has a nuclear weapons program, but there is no reliable proof that it can produce nuclear weapons."

U.S. analysts are more bullish on North Korea's odds of pulling it off. "Per capita, the DPRK has probably invested as much in its weapons programs as the Soviets did," notes a State Department official. Erring on the side of caution, perhaps, the CIA estimates that North Korea has made as many as eight plutonium bombs. At the Yongbyon nuclear facility last January, North Korean scientists showed what appeared to be plutonium metal to a delegation including Siegfried Hecker, senior fellow and former director of Los Alamos National Laboratory in New Mexico (*Science*, 23 January, p. 452). But the jury is out on whether Korea's nuclear specialists have devised the precise configuration of explosives needed to get a plutonium bomb

selling Korea everything from mining machinery to Volvos, but even then North Korea wasn't playing by the rules. "At first they paid for some of the stuff they bought, then they stopped paying and accumulated a huge debt," the diplomat says. Trade with Sweden and the rest of Western Europe dwindled.

Korean science, meanwhile, was flush with cash into the 1970s, researchers say. "We were extremely well supplied by the state," says Choe Sung Ho, director of ASK's Institute of Microbiology. During the Cold War, North Korea dispatched hundreds of its top scientists for training in Soviet labs, including the Joint Institute of Nuclear Research in Dubna, as well as labs in the Eastern Bloc. But tensions between North Korea and the Soviet Union were always near the surface, especially as Kim's government in the 1970s began weaving a mythology about his life and the nation's history. "We would tell them to cut down on the propaganda and fantasy—and that was coming from the Soviet Union!" exclaims Mikheev, who served as a diplomat in the Soviet embassy in Pyongyang in the early 1980s and returned to North Korea for a visit last July.

Relations quickly soured after the Soviet breakup, to the extent that in the early 1990s several dozen Russian missile experts were arrested as they tried to travel to Pyongyang, Mikheev says. "Now there are no DPRK scientists working in Russian institutes," says an official with Russia's Ministry of Science. North Korea's on-again, off-again relationship with China became more important, and scientific ties between the two countries increased. "Our country was in a very difficult situation," says Ju Song Ryong, director of the Central Information Agency for Science and Technology in Pyongyang. "But our Great Leader set forth a 'science first' policy," he says—raising science's prestige, if not its funding, to that of the military. The North Koreans also cottoned on to one skill essential to entering the scientific mainstream. "In the past, we older scientists learned Russian," says Choe. "Now young people have come to know that English is important for science."

Nurture then nature. After raising acacia shoots in culture for a month, Un Song Gun's group transfers the hardiest specimens to the field, where the trees grow about 2 meters per year.

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After the elder Kim's death in 1994 and a series of devastating floods and crop failures, Kim Jong Il, echoing his father's words, turned to his scientists for a way out of the country's miseries. Kim has called science and technology one of three pillars for building a prosperous

to fission. A global surveillance network assembled under the Comprehensive Test Ban Treaty has not picked up the telltale seismic signals of a successful nuclear test in North Korea. "As far as we know, no testing has been done," says Tetsuya Endo, vice chair of Japan's Atomic Energy Commission. (Western experts are, however, keenly watching a recent flurry of activity at suspected test sites in North Korea.)

More alarming is the prospect of North Korea enriching uranium, analysts say, because it's easier to get a uranium bomb to undergo fission. That concern triggered the current crisis. In October 2002, U.S. diplomats confronted their North Korean counterparts with evidence that the reclusive state was secretly attempting to enrich uranium. Although the U.S. dossier has not been made public, elements have been leaked. For example, U.S. officials claim that A. Q. Khan, the father of Pakistan's nuclear program, has admitted to having provided North Korea in the 1990s with nuclear technology, including a design for a gas centrifuge for concentrating weapons-grade uranium. North Korea also may have imported equipment for an enrichment facility through Korean-run export companies in Japan. "We have a history of stopping exports from Japanese companies," says Mori.

Japanese and U.S. officials who spoke with *Science* acknowledge that they have few clues to the location of the uranium-enrichment program or how advanced it is. "No one has seen it," says Mori. North Korean officials steadfastly deny that a uranium program exists. That has thrown up a hurdle at the six-way talks, in which Japanese and U.S. officials have insisted that the uranium program's dismantlement is integral to any deal. "It's a major stumbling block," says Mori.

Plutonium production line. The reprocessing facility at Yongbyon.

Despite the political barriers, U.S. nonproliferation officials see a tantalizing possibility in these negotiations. They may offer a way to redirect North Korea's nuclear scientists—and perhaps even its cadre of researchers involved in biological and chemical weapons R&D—to peaceful research. According to one U.S. official, there may be several hundred North Korean weaponeers "of interest."

Still unknown is whether the North Korean government will echo the unofficial suggestion that it is ready to abandon its nuclear program for clean energy technologies. In addition to describing this possibility, the unofficial envoy—who spoke with *Science* at an unusual meeting arranged by other diplomats—reiterated old themes: that North Korea desires bilateral talks with the United States to solve the nuclear issue and that it is ready to discuss diplomatic relations between the two countries.

A State Department official told *Science* that the U.S. government is preparing to raise the amount of aid offered to North Korea in the next round of talks in exchange for dismantling the uranium program. The prospect of a better deal would strengthen the hand of advisers to Kim Jong Il who advocate cooperation with the United States, argues Mikheev. But the U.S. commitment will need to be solid, he says, adding that North Korea is unlikely to settle for anything less than an agreement ensuring the present government's survival. After all, its nuclear program is its only real bargaining chip. "Without nuclear weapons North Korea would be just a poor country with no attention," asserts Endo. "Kim Jong Il is fully aware of that." **–R.S.**

nation, the other two pillars being ideology and the military. In a 1997 treatise on *Juche*, he proclaimed the necessity of "expanding and developing scientific and technical exchanges with different countries and introducing advanced science and technology from other countries." The policy statement reaffirms a core *Juche* principle: "There's no restriction on North Koreans using the outside world for their own betterment," says the Swedish diplomat.

Ambiguity about whether Kim's guidance meant that North Korean scientists had carte blanche to reach out to the West or whether it implied a more furtive acquisition of knowledge from abroad prevented any significant movement, however. But in recent months, government missives have clarified the situation: ASK and university researchers have explicit orders to bring in foreign grants whenever possible. Still, lack of contacts—especially e-mail links—and funds continue to present major barriers.

Today North Korea claims to have 1.8 million "intellectuals," including an estimated 100,000 working scientists. But the state has the means to pay only utility bills and meager salaries. ASK scientists earn between $20 and $40 per month at the official exchange rate, but one-tenth that at the black-market rate. Institutes have scant funds for foreign travel, and these are largely reserved for train tickets to China for training courses.

Even so, a substantial proportion in this favored occupation is doing better than their compatriots, who in rural areas must eke out a hardscrabble existence. For residents of Pyongyang, at least, the Communist-style food distribution system introduced by Kim Il Sung remains intact, providing monthly rations of staples such as rice, bread, and eggs. "We know very little about who's getting what and why," says the Swedish diplomat. Scientists still receive rations, says an ASK official.

A party policy paper published last month in the state newspaper *Nodong Sinmun* reaffirms science as a high priority, declaring: "It is our party's unwavering determination and will to raise the country's science and technology to the world standard in a short period of time and, based on this, to open up a phase of turnaround in the construction of a powerful state." *Juche* or no, North Korea can't do this alone. Many factories are shuttered due to a lack of energy and raw materials, and there is little private enterprise. "The North Korean economy is in a very deep crisis," says Mikheev.

Still life. The Biology Branch's museum room displays pickled critters from across the country.

Science for the masses

North Korea's homegrown civilian science, at least the portion that *Science* was allowed

CREDITS (TOP TO BOTTOM): SPACEIMAGING INC.; R. STONE/*SCIENCE*

to glimpse, is mostly tied to areas of potential economic growth. A plant genetics lab has introduced a line of virus-resistant potatoes into commerce and is trying to transfer insect-resistant *Bacillus thuringiensis* genes into corn, rice, and oilseed rape. Chinese agricultural scientists have come to North Korea to collaborate on transgenic experiments, including field trials, says Kim Song Jun, director of the Branch of Cell and Gene Engineering. Another team is growing acacia shoots in tissue culture to clone the hardiest trees. "Many trees were cut down freely during the Korean War and after the war," says Un Song Gun, chief of the institute's tissue culture lab. "We'd like to reforest entire mountains" with both imported and native acacia varieties, he says.

Other efforts aim to improve public health. One team, for example, is cloning the gene for erythropoietin, a hormone that stimulates the body to make blood cells, with a goal of infusing the protein in anemic patients. So far they have succeeded in expressing the gene in Chinese hamster cells. Another group extracts tetrodotoxin from puffer fish for use as a drug to treat tuberculosis, a particular scourge in North Korea; plans are afoot to export the preparation to China and Vietnam. Malaria is another woe, with approximately 300,000 cases per year. And an untold number of children are malnourished. The Swedish diplomat puts it bluntly: "A very ordinary disease in the West will kill you here. If you're malnourished and get the flu, you're dead."

The World Health Organization aims to combat this health crisis by stepping up its support to North Korea. Until last year WHO was mainly providing medicine and equipment to North Korea's Ministry of Health. "The plan now is to provide more technical expertise," including assistance for training North Korean public health specialists abroad, mainly in Thailand, says Diego Buriot, a WHO special adviser who traveled to Pyongyang last year to initiate these discussions. He and other WHO officials have also floated the idea with their North Korean counterparts of organizing an international conference in Pyongyang to review communicable disease surveillance systems in the region, with a goal of improving information exchange.

Also hoping to make inroads into easing the country's health woes is ASK's Institute of Microbiology, which specializes in medicinal polysaccharides. One preparation, *Jangmyong*, is a pair of polysaccharides derived from mushrooms that grow on pine trees. It is touted as an immune-system booster—it's claimed to rev up T cell production in bone marrow—for curing everything from brain cancer to epilepsy. *Jangmyong* is now being exported to China, Japan, Malaysia, and South Korea, says its developer, Mun Ho of the Institute of Microbiology. Further work is stymied by a shortage of supplies, says Mun: "There are many polysaccharide standards in the Sigma catalog we would like to have, but there's no way to buy them."

Closer to the ethical fringe, the North Korean government has a policy of administering human growth hormone to all children, aged between 12 and 15, who are deemed "unusually short"—less than 140 centimeters tall. The injections add about 1 centimeter in extra growth per year, says Kim Song Jun. His institute is conducting clinical trials of growth hormone for use in promoting health, from improving metabolism and softening skin to promoting faster recovery after operations.

One giant leap. Asia expert Vasily Mikheev, an advocate of science diplomacy, with two Korean colleagues at the Goethe Institute's new reading room in Pyongyang.

The pride of ASK's Biology Branch is its claimed cloning breakthrough. The impetus was a visit by Kim Jong Il to the Institute of Experimental Biology on 13 June 1999. Kim's appearances at institutes, and far more frequently at bases of the People's Army, are portrayed as opportunities to "give guidance" to his people. During the 1999 visit Kim "came to know that we lacked equipment," says branch president Son Kyong Nam; Kim later "personally" provided the institute's Cloning and Reproductive Technology Center with a centrifuge, microscopes, and other equipment. Also crucial were key articles on cloning from overseas sent back by North Korean officials. Armed with equipment and knowledge, the team—"almost all of them young scientists," says Kang Gyu Chol, scientific and technical counsellor at the DPRK Embassy in Moscow—set to work.

After producing cloned rabbits using embryo transfer, the researchers claim they succeeded through trial and error in the far more difficult technique of somatic cloning. The first such pair of rabbits, cloned from fibroblasts derived from a 15-day-old fetus, was born on 1 July 2002. If true, that would mean that the group was the second in the world to clone rabbits from somatic cells. The first North Korean pair proved fertile, and the lab has since produced two more pairs, the latest born in August 2003. Ri notes that Kim Jong Il personally thanked the group for its breakthrough. The cloning center also has a keen interest in launching a stem cell research program with Western assistance, with the ultimate aim of developing treatments for paralysis and kidney diseases.

Breeding supergoats

The experimental farm where ASK intends to raise its agbiotech game lies an hour's drive east of Pyongyang. Our journey begins on a six-lane highway leading out of the city. Traffic is heavy—but it's not vehicular. Scores of people, adults and children, some bedraggled, are walking along the shoulders of the quiet road; public transportation outside Pyongyang appears to be virtually nonexistent. Occasionally we pass a farmer driving an ox yoked to a wooden plow. After about a half-hour on the highway we turn off onto a bumpy dirt road that wends through a broad valley lined with jagged hills, past cornfields and rice paddies and the occasional long, wooden sign with white letters on a red background extolling the glory of Kim Il Sung. It's the rainy season, and in a few spots the rivers have overflowed their banks, washing out the road. Our van forges ahead.

We pull into one of ASK's experimental farms. "This doesn't look like much now," says Son, nodding to a collection of spare wooden outhouses and pens. He has big plans to transform the farm into a stock breeding center for the creation of herds of supergoats using embryo transfer and someday, he hopes, cloning. "This is our base for the introduction of embryo transfer throughout the country," he says. The state recently approved the construction of a facility to house several hundred elite goats—breeds that ASK hopes to import with Western money—as well as labs for reproductive technologies.

Mountains cover roughly 80% of North Korea, leaving little arable land, and the country has suffered from crop failures in recent years. Thus in the late 1990s the

CREDIT: R. STONE/*SCIENCE*

The Ultimate, Exclusive LAN

PYONGYANG—North Korea's success in integrating itself with the rest of the world, beginning with science, could hinge on whether it opens up lines of communication to foreign information and ideas. But that's a risky notion in this isolated country.

Leader Kim Jong Il inherited from his father, Kim Il Sung, a distrust of alien influences and an enthusiasm for technology. This skittishness is embodied in North Korea's strategy for importing and disseminating technology. Spearheading the effort is the Central Information Agency for Science and Technology (CIAST). By the mid-1990s CIAST had developed its own database engine and in 1997 began installing a national computer network—a simulation of the World Wide Web that's unconnected to the outside world.

North Korean Webmaster. Ri Hyok's team at CIAST has built a formidable countrywide computer network.

Ri Hyok, who heads CIAST's Computer Center, shows off the home page for the countrywide intranet (one your browser will never find). It's as slick and easy to navigate as Yahoo. It provides access to tens of millions of records, according to Ri, including several online North Korean journals, a science encyclopedia, and a wealth of analytical information compiled by a 600-strong staff on topics from agriculture and construction to the writings of Kim Il Sung. The system, called "Kwangmyong," or "light," was upgraded recently with fiber-optic links to major North Korean cities and now has approximately 10,000 subscribers, says Ri. Subscriptions are free; users only pay telephone charges. "We don't know how their intranet works," confesses a Swedish diplomat. CIAST says it raises funds through the export of software, such as a Japanese-Korean translation program now being sold in Japan and other countries.

Ri says he and his CIAST colleagues are keen to boost capacity and access Western literature electronically. That would be straightforward if they could tap external Web sites. Asked whether there are plans to connect to the Web, Ri offers a strained smile. "No plans," he says.

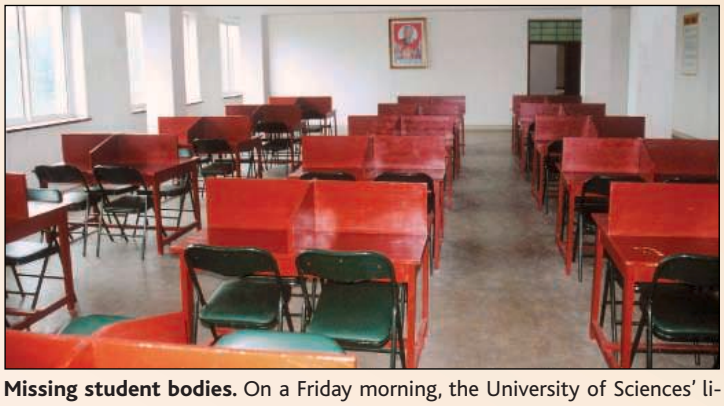
The Academy of Sciences has external e-mail—a single address for the entire organization. (Internet access in North Korea is restricted to a small, trusted fraction of the population.) If CIAST were to make portions of its Web site available to the outside world, it could solicit external funding or collaborators. But for now that's not feasible, says Ri: "It's an administrative issue."

Access to up-to-date scientific information through printed books and journals is also lacking. On a leafy campus a half-hour's drive south of Pyongyang, the Academy of Sciences runs an intellectual hothouse—the University of Sciences, part of its Unjong regional branch. This university, with a student body of 3000, cherry-picks "genius" students from throughout the country, of which 60% continue in postgraduate studies, say university officials. Faculty members, including about 100 Ph.D. lecturers and professors, also maintain laboratories that work on everything from the production of cisplatin for cancer therapy to studies of laser-ignition of dynamite and a magnetometer for detecting hidden weapons. The university's Korean-Chinese Friendship Laboratory features a laser setup, donated by China more than a decade ago, for the study of plasmas.

Yet a glimpse of the university's library shows how disconnected the students and faculty may be. It's a Friday morning; classes are in session. But the reading room, desks aligned to face the visage of Kim Il Sung, is deserted except for a solitary librarian. When asked for examples of the library's English-language science journals, the librarian disappears momentarily into the stacks, a room no bigger than a 7-11 store. She retrieves a half-dozen issues, bearing the treasures to her desk. They look old, and they are. An issue of the *Journal of Quantum Electronics* from 1977. *Genetics*—1981. *Microelectronics*—1973. A generation of science has passed them by.

Some foreign organizations have tried to help fill the information void. For example, the Asia Foundation, with headquarters in San Francisco, has provided more than 70,000 books and journals to the Grand People's Study House, or central library, Kim Chaek University of Technology, and Pyongyang University for Foreign Studies, all of which are in Pyongyang. And the Goethe Institute of Berlin last June opened a reading room with some scientific literature in the Chollima House of Culture in Pyongyang. Still, as the Swedish diplomat observes, the vast majority of North Koreans "have very little idea of the outside world."

Sometimes the government's efforts to catch up are too quick for its own comfort. Last year it launched a cell phone network, issuing about 10,000 handsets, estimates a Russian diplomat. He says that within a few days after the deadly train explosion at Ryongchon last April, which occurred several hours after Kim Jong Il's train from China passed through the station, the government began recalling most of the phones. The network is still operating—diplomats in Pyongyang say they use it for their cell phones—suggesting that some North Korean elites have kept their handsets. It's unclear why the government clamped down, although the diplomat speculates that it is nervous about "horizontal communication." Perhaps, he says, in the hours after the Ryongchon accident, the cell network was abuzz with gossip over whether the explosion was an assassination attempt, an idea that has since been discounted.



Missing student bodies. On a Friday morning, the University of Sciences' library does not appear to be a top draw.

"To achieve a free flow of people and knowledge, the society has to be ready," notes Mikio Mori of Japan's Ministry of Foreign Affairs. For now North Korea's intellectual community is extending a hand to the outside world with trepidation, unsure of who will grasp it or how new contacts may shape the country's future.

–R.S.

CREDITS: R. STONE/*SCIENCE*

government made it a policy to raise the productivity of goats and rabbits, which are adept at grazing on rough terrain. (As with all major policy decisions, Kim Jong Il is credited with the inspiration to breed better goats.) Native goats produce between 150 and 200 liters of milk a year, whereas a Swiss breed, Saanen, can churn out much more. ASK has fewer than a dozen Saanen and wants to purchase a couple of hundred next year.

After conquering embryo transplantation in mice in 1988, Son says, a year later North Korean scientists succeeded with artificial insemination and surgical transplantation in goats. Son says Korean scientists hope to improve their skills through training and ultimately move to nonsurgical embryo transfer. By transferring embryos of Saanen or other well-bred species into native goats, they hope to multiply their herd of elite goats to more than a quarter-million by 2010. Also on the agenda is impregnating indigenous goats with Saanen sperm, with the aim of breeding more than 4 million hybrids in the next 5 years.

"The overall goal is to meet the needs of our people for meat and milk by developing and applying new reproductive techniques," Son says. But research materials are in short supply. "We feel the lack of infrastructure," says Son, who notes that his researchers have had to improvise, for example by using special methodology for freezing and preserving sperm with Soviet-era equipment. "Importing new equipment would not be a problem," he says. "The only problem is money."

Well, not quite the only problem. One expert with a U.S. nonprofit that has worked extensively with North Korea to provide agricultural assistance says that the goat-breeding proposal "is typical of the ideas we have seen and received: too much, too fast, an emphasis on high-tech solutions rather than basic management, and an underestimation of the technical resources needed to initiate the project." He cites a cautionary tale from his own experiences in Korea. A few years ago the organization shipped purebred boar and bull semen to the DPRK Academy of Agricultural Sciences to upgrade their herds. The academy failed to inseminate any cows and produced only two small litters of pigs. Although it was unclear where the problems lay, the expert concludes that "they were clearly substantial for such an abysmal result." Nevertheless, he says, aid organizations should be ready "to support something reasonable in this area of work." He envisions a project that could involve training a handful of North Korean scientists abroad to bring them up to speed before providing equipment and reagents. "Otherwise it will just get wasted," he says.

Goats are not the only animal on the menu. In the early 1990s, ASK researchers collaborated with a Czech team on embryo transfer in cattle, and they hope to try cloning as well. On a hill a few hundred meters away, workers are laying cinderblocks for the facility to conduct such experiments on goats and cattle. "Our government is paying much more attention now to this site, because it's a place where we can link scientists and farmers," says Son. "At the end of this year it will be ready."

One thing that would help is a better road. On the way back to Pyongyang our van, avoiding a washed-out section of road, got stuck in a gully. A passing unit of the People's Army, mostly boys in their late teens, appeared out of the blue and pushed the van out. I decided to keep my mouth shut, recalling my visit the previous day to the War Museum in Pyongyang, where I was told that America's "puppet regime" in the South attacked the North to start the Korean War in 1950—exactly the opposite of what the rest of the world is taught. There I had heard a litany of war crimes and cowardly acts that the North Koreans attribute to U.S. forces. "You can understand why we hate Americans," one of my escorts had explained in a rectitudinous tone. If it weren't for the unwitting assistance the soldiers provided to the enemy—North Korea and the United States are still technically at war—we might have been stuck there all night. I wondered, though, whether U.S. scientists would have to bear such enmity for the sake of a joint project.

Window to the world. The Asia Foundation has donated thousands of books and journals to the Grand People's Study House (center).

Promises and perils

Nearly a half-century ago, when the Soviet Union in 1957 launched Sputnik, the world's first satellite, many observers feared that the Soviets had overtaken the United States in the physical sciences. But because there was virtually no contact between the Soviet and U.S. scientific communities, westerners could only speculate. By 1959, the U.S. National Academy of Sciences (NAS) had struck up a scientific exchange program with its Soviet counterpart, in which approximately 20 specialists from each superpower visited the other. The U.S. side quickly gleaned that fears of Soviet scientific dominance were overblown. "The myth of the superiority of Soviet science would not have spread so far if scientific contacts with the Soviet Union before 1957 had not been so few," concluded an influential 1977 review of the exchange program by an NAS panel chaired by political economist Carl Kaysen of the Massachusetts Institute of Technology. The exchanges, the Kaysen report concluded, achieved "striking success" in learning about Soviet strengths in science and generally improving U.S.-Soviet relations.

The model may work for North Korea as well, but—as with the Soviets—there are risks and benefits to consider. On the one hand there are tantalizing opportunities to expose Korean scientists, some of whom may exert influence on future governments, to Western ideas. Engaging them could strengthen the positions "of those in North Korea who hopefully will be able to support reforms if, or when, they start," says Mikheev.

But on the other side are concerns about so-called dual-use technologies: the potential diversion of equipment from benign research to weapons R&D. That could doom some projects before they get off the ground. "Strong control is needed over what kind of equipment and supplies will be provided to North Korea," says Mikheev. The Institute of Microbiology, for example, would like to expand its work on medicinal polysaccharides. "We need fermenters," says institute director Choe, who notes that quality fermenters can cost thousands of dollars. But fermenters, a major quarry of weapons inspectors in Iraq, could be diverted for use in cooking up pathogens.

Dual-use concerns boil down to a dearth of trust. "We run into a problem: Who are we talking to?" says the Swedish diplomat. "Is it really a biochemist, or is it a colonel in the army? Or the head of their bioweapons program? We have to try to find programs that will not strengthen their military or boost their economy at this stage." In negotiations on a cooperation agreement last year with ASK, Russian officials steered discussions away from activities that could involve dual-use technologies. "We agreed to exchange open, unclas-

CREDIT: R. STONE/*SCIENCE*

sified scientific literature," says the Russian science ministry official. North Koreans, too, are wary, assuming that foreign visitors have government connections, no matter what their affiliation.

Overcoming such suspicions will require that joint projects be fundamentally benign or benevolent. "Projects should be based on humanitarian needs," says Shunichi Yamashita, chair of the Atomic Bomb Disease Institute at the University of Nagasaki, who has worked extensively on scientific aid projects in the former Soviet Union. One area that gets top marks as a confidence-builder is the environment. In a rare joint effort with South Korea, for example, ASK's Biology Branch is translating into English a compendium on North Korea's roughly 4000 native plants.

Agricultural exchanges too have borne fruit. For example, the American Friends Service Committee (AFSC), a Quaker-founded nonprofit, has brought North Korean delegations to the United States, China, and Vietnam to study topics such as rice and corn breeding, potato seed generation, and poultry production. "The North Koreans can freely interact with researchers and have an extremely valuable opportunity to acquire copies of journal articles, research reports, and other published information," says AFSC program representative Randall Ireson, who has traveled to North Korea. The study tours, he says, have resulted in, for example, more economical feed formulation for poultry and swine, better rice breeding, and the use of legume cover crops for improving soil fertility.

University exchanges also could build bridges. Since 2002, the German Academic Exchange Service (DAAD) has sponsored exchanges between German universities and several institutions in Pyongyang, including Kim Il Sung University, ASK, and the Academy of Agricultural Sciences. Academic relations "are improving steadily," says DAAD's Ursula Toyka-Fuong, with plans in 2005 to invite two Korean researchers for long-term stints in Germany, as well as offer short-term scholarships for up to 6 months to several promising young scientists.

A similar exchange program with Kim Il Sung University was launched last year by Far Eastern National University in Vladivostok, Russia, which already had South Koreans among its student body. "At first the North Koreans and South Koreans got into a lot of fights. It was a big problem for us," says Valery Dikarev, vice president for international affairs at Far Eastern. "But at the end of the year the South Koreans saw off the North Koreans at the train station—and they all were crying." Dikarev visited Pyongyang in July as part of a delegation to expand Far Eastern's academic agreement with Kim Il Sung University to include joint research; initial projects will probe the biochemistry of traditional Korean medicines. Kim Il Sung University's economics institute, meanwhile, has asked the Swedish Embassy to help devise improved course materials. "I find it intriguing that we can change their curriculum," says the Swedish diplomat.

The United States sees an entrée as well. Syracuse University in New York and Kim Chaek University of Technology in Pyongyang have exchanged delegations and are working to establish twin laboratories for joint research on integrated information technology. The project, funded by the Henry Luce Foundation and the Ford Foundation, suggests "the possibility of serious, sustained, and mutually beneficial collaborations between an institution in the DPRK and one in the U.S.," according to a recent status report from the team.

Another budding initiative is a U.S. committee now being organized with members from some 50 nonprofits, universities, and other organizations to engage North Korea in a range of areas, including science. And NAS and the U.S. National Academy of Engineering (NAE) are discussing ways to cooperate with ASK on energy and agricultural projects. The discussions were initiated in Pyongyang last January by Siegfried Hecker, senior fellow and former director of Los Alamos National Laboratory in New Mexico and a member of NAE's council. Hecker is now serving as a liaison between the U.S. and North Korean academies.

"I'm convinced there are interesting research programs in North Korea, including those interesting to world science. We just have to discover and support them," says Zurab Yakobashvili, director of the International Centre for Scientific and Technical Information in Moscow, which is helping to organize a symposium next spring to bring together North Korean and Western scientists to discuss joint projects in areas such as biotechnology, information technology, and materials science. North Korea's rank-and-file researchers are raring to go. "We have many young scientists who are very well qualified," says Choe. "If even one is able to travel to Europe, we can learn a lot. But now we're just sitting here."

Mikheev, who has long advocated a cautious approach to North Korea, views scientific cooperation as an exceptionally constructive tool for engaging the DPRK. "From a strategic perspective, scientific diplomacy seems to be very important to provide peace on the Korean Peninsula," he says. Forging contacts with key individuals could well help shape North Korea's future and give rise to the exhilarating possibility of bringing an entire nation in from the cold.

—RICHARD STONE

Raring to go. Many scientists want to go abroad for training, says Choe Sung Ho of the Institute of Microbiology. "But now we're just sitting here."

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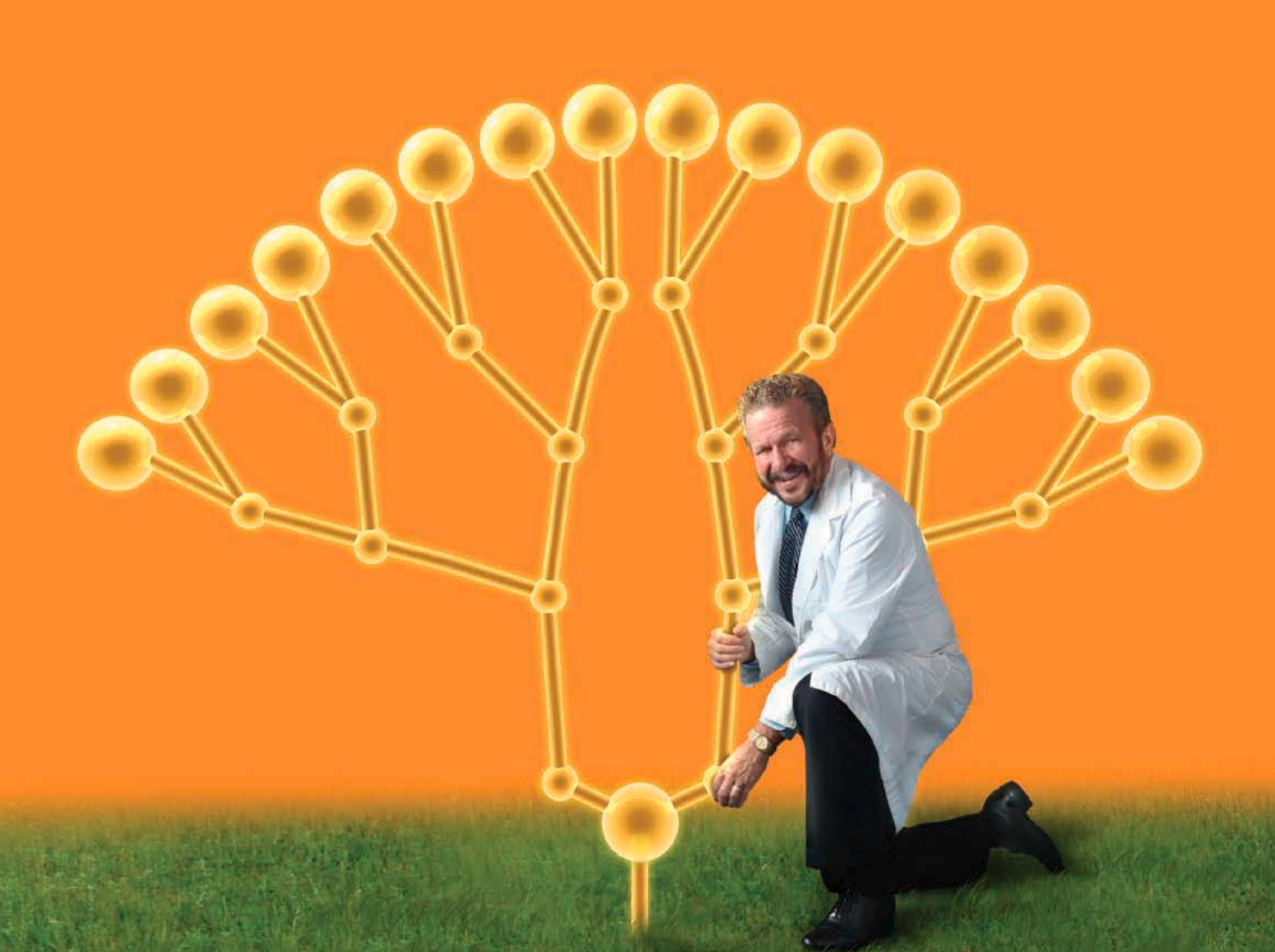

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In Mass Extinction, Timing Is All

A new, apparently improved, way to date the greatest mass extinction points to a volcanic cause but fails to resolve geochronologists' long-running differences

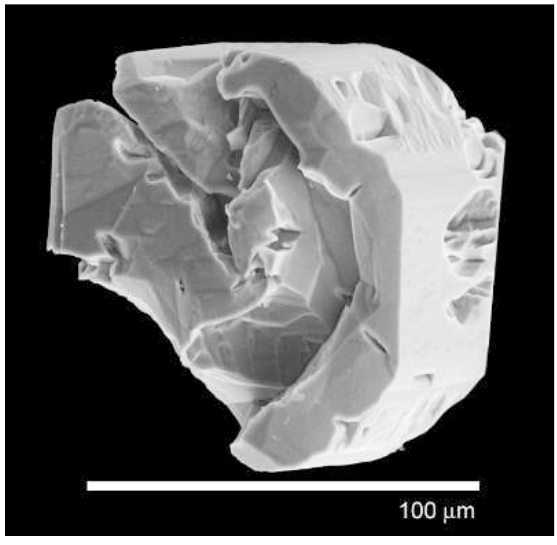
For earth scientists trying to lay the blame for the all-time greatest mass extinction some 250 million years ago, the secret is in the timing. The professional timekeepers—the geochronologists—are trying to place a volcanic catastrophe at the moment of the extinction, thus linking cause and effect to explain an event that wiped out 95% of animal species on Earth.

But nailing down the time of the Permian-Triassic (P-T) extinction has revealed problems in the often competitive business of geochronology. P-T daters must draw their conclusions from vanishingly small isotopic remains of radioactive decay. For years, different laboratories using uranium-lead radiometric dating—the gold standard of geochronology—have been getting entirely different ages for the P-T extinction.

On page 1760 of this issue of *Science*, Roland Mundil of the Berkeley Geochronology Center in California and colleagues weigh in with their latest P-T age using a new way of preparing samples for uranium-lead dating. By their reckoning, the extinction and the largest volcanic eruption of all time are older than thought, but coincided precisely. "It's an impressive piece of work," says geochronologist Michael Villeneuve of the Geological Survey of Canada in Ottawa. The new treatment seems to remove much of the subjectivity of traditional approaches, but still, "all dates are interpretations," Villeneuve notes. "It needs a bit more proving out."

Uranium-lead dating seems straightforward enough. The analyst simply crunches a rock, picks out microgram grains of the mineral zircon, grinds off an outer layer, dissolves the remaining grain in acid, spikes the solution with a calibration standard, and measures the amounts of four isotopes using mass spectrometry. Two are isotopes of radioactive uranium that have not yet decayed, and two are picogram quantities of lead isotopes that have accumulated from the steady decay of the uranium since the zircon crystallized in magma. The ratio of each uranium isotope to its decay-product lead isotope tells how long each of the two radioactive clocks has been running and thus how old the rock is.

Geochronologist Samuel Bowring of the Massachusetts Institute of Technology and colleagues followed just such an approach to date rocks bearing fossils from the time of the P-T extinction in southern China. Analyzing zircons from nearby layers of volcanic ash, they got an age of 251.4 ± 0.3 million years (*Science*, 15 May 1998, p. 1039). And late last year Sandra Kamo of the University of Toronto, Canada, and colleagues published a uranium-lead date of 251.4 million years—right on Bowring's P-T age—for the eruption of the massive Siberian Traps, thick layers of ancient lava that once covered millions of square kilometers (*Science*, 21 November 2003, p. 1315).



Better for it. Hot acid has removed degraded parts of this zircon that would have skewed its apparent age.

Mundil, however, doesn't believe that either the eruption or the extinction happened that recently. He thinks Bowring engaged in "arbitrary data culling" by throwing out more than half his zircon ages before averaging the rest of them together. But Bowring says his choices were judicious, although "necessarily somewhat subjective." In some of his zircons, the two different uranium-lead ratios gave different ages, suggesting that lead had leaked out of those zircons during the past quarter-billion years. And other zircon ages looked distinctly old, as if those zircons had crystallized earlier than the rest and had later gotten mixed in with them. By taking into account how volcanic ash beds are stacked around the rock layer that shows the extinction, Bowring believes he can confidently select the reliable zircon ages and discard the rest.

Mundil set out to take this "picking and choosing" out of uranium-lead dating. Over the years, researchers had tried various pretreatments to get rid of the parts of a zircon that had lost lead. To prepare new samples from southern China, Mundil and colleagues adopted a technique recently developed by James Mattinson of the University of California, Santa Barbara. They baked the southern China zircons at 850°C for 36 hours and then leached them with hydrofluoric acid under pressure at 220°C for 16 hours, with the intention of removing the parts most weakened by radiation damage.

This rugged pretreatment narrows the range of zircon ages from a single ash bed from about 20 million years to a few million years, with no picking and choosing. Of the 79 zircons dated in the P-T study reported in this issue, the researchers discarded only three, all for being obviously too old. Their age for the P-T extinction is then 252.6 ± 0.2 million years—about a million years older than Bowring's age but coincident with a decade-old argon-argon radiometric age for the Siberian Traps that Mundil and his colleagues—after making a 2-million-year correction to it—prefer over Kamo's uranium-lead age.

The new preprocessing technique "is very promising," says Drew Coleman of the University of North Carolina, Chapel Hill. "It appears to be very fruitful." Bowring agrees. "This is a step in the right direction," he says. "Mattinson's annealing is the big breakthrough, though I have no idea why it works." But Bowring points to the later date that his group estimated for the P-T extinction in China and Kamo's group independently got for zircon and other minerals from the lavas of the Siberian Traps. Mundil hasn't explained how subjective interpretation could have produced such a coincidence, he says.

What uranium-lead geochronologists need now, all agree, is more cooperation. "They've been competitive and secretive for decades," says geochronologist Randall Parrish of the British Geological Survey in Keyworth, U.K. "There's not enough cooperation among workers seeking out best practice, but we're going to hash out a lot of these issues in October." That's when uranium-lead and argon-argon daters will gather near Boston under the auspices of the new EARTHTIME program for a frank and open discussion of all those little details that don't make it into the literature.

–RICHARD A. KERR

CREDIT: R. MUNDIL/BERKELEY GEOCHRONOLOGY CENTER

Biosecurity Goes Global

The 2001 anthrax letters triggered a strong U.S. response. Now the rest of the world is starting to take biosecurity more seriously—but not necessarily by adopting the U.S. approach

Three years ago, the small number of life scientists using the term "biosecurity" were talking about ways to keep diseased crops and livestock from crossing national borders. Then came the fatal October 2001 anthrax letter attacks against several U.S. targets. In short order, thousands of U.S. scientists were confronted with an avalanche of new and often unpopular rules designed to keep potentially dangerous pathogens and toxins away from bioterrorists. Researchers who break those rules could face significant criminal penalties.

Despite these aggressive steps on the home front, U.S. officials readily acknowledged that unilateral action was insufficient and that the world needed to form a united front against increasingly sophisticated biotechnologies. But many nations were skeptical of the threat. They also doubted the value of what critics call "the guns, guards, and gates" approach to biosecurity. The result, says Reynolds Salerno, a biosecurity expert at Sandia National Laboratories in Albuquerque, New Mexico, has been "tremendous confusion and concern in the international life sciences community about biosecurity."

That confusion may be giving way to cooperation, however, as an increasingly global effort to define and implement biosecurity is gaining speed. Nations are moving to pass new biosecurity laws, while public health and security experts are hammering out voluntary biosecurity guidelines and debating "codes of conduct" for life scientists. Many countries are thinking about looser rules for less risky agents than in the United States, which critics say has imposed a one-size-fits-all approach, and few are likely to require the extensive criminal background checks carried out by U.S. agencies.

The new world order may not resemble the U.S. model. But like it or not, life scientists worldwide are about to become much more familiar with the term biosecurity.

–David Malakoff

Spreading the word. U.K. officials are preparing to host a Bioweapons Convention–related summit in October 2005 on "codes of conduct" for life scientists who work with potentially dangerous pathogens and biotechnologies. Although few believe that such codes will deter evildoers, advocates say they can play an important role in raising awareness of biosecurity. This winter, academic and industrial scientists will gather in Washington, D.C., to sign a pledge to help prevent the misuse of biological research—a theme also stressed in a new public relations campaign (left) by the International Committee of the Red Cross (www.icrc.org). Such efforts are "a way to encourage dialogue," says Michael Moodie of the Chemical and Biological Arms Control Institute, an organizer of the Washington meeting. In the meantime, the Federation of American Scientists and other groups are preparing biosecurity course materials for undergraduate and graduate students.

Whose resolve? Last April, the United Nations Security Council adopted Resolution 1540, which expresses "grave concern" about bioterrorism and directs U.N. members to enact tough controls on potential bioweapons. The resolution is intended to help close legal loopholes in dozens of nations—including some with growing biotech industries—with laws that don't cover all the bases. "They are now obligated to build the legal framework needed for effective biosecurity," says Barry Kellman, a law professor at DePaul University in Chicago, Illinois. Critics, however, see the measure as a U.S.-backed gambit to sidestep efforts to strengthen the Biological and Toxin Weapons Convention, which is in limbo until at least 2006.

Biocrime fighters. Interpol, the International Criminal Police Organization, has launched a 2-year effort to train police in its 181-member countries on biosecurity and fighting bioterrorism. "You'd be amazed at how little the average police chief knows about the subject," says Barry Kellman of DePaul University, who is involved in the project, which is funded by the Alfred P. Sloan Foundation. One goal: to teach investigators how to avoid lumping legitimate researchers in with the biocriminals.

Self-help book. Early next year, the 192-member World Health Organization (WHO) plans to unveil its first-ever set of international biosecurity guidelines. The consensus how-to guide, currently in draft form, should help "clear up a lot of confusion ... by clarifying best practices and minimum standards for keeping pathogens secure," says Brad Kay, a WHO biosafety expert in Lyon, France.

But implementing the voluntary standards is another story. Many poorer nations won't want to divert precious public health funds to security, and WHO has meager resources to help out. It also isn't clear what would happen to labs that don't meet the standards. "WHO has no mandate to become a global enforcer," says Kay.

In the United States, meanwhile, a team of government and academic researchers is writing a new biosecurity chapter for the "bible" of lab safety, *Biosafety in Microbiological and Biomedical Laboratories*.

Center of expertise. The United States and Europe are spending more than $90 million annually to help Russia secure its sprawling former bioweapons complex and employ an estimated 6000 former bioweapons scientists. But efforts to attract investment from foreign biotech and drug firms have had mixed results, and some critics say more needs to be done to prevent ex-Soviet pathogens and weapons experts from leaking into the black market. "Biosecurity is about limiting the spread of expertise, too," says Amy Smithson, a nonproliferation specialist at the Center for Strategic and International Studies in Washington, D.C.

Asia alert. Asian Pacific leaders pledged last year to get tough on biosecurity—in part due to fears that their rapidly growing biotech industries could attract regional terrorist groups along with investors. "Singapore views this threat with grave concern," Deputy Prime Minister Tan Keng Yam said at a biosecurity conference held in the city-state earlier this year. China, meanwhile, has ratcheted up export controls and is examining both its biosafety and biosecurity rules in the wake of the SARS epidemic and several lab accidents.

Lessons learned. The Republic of Georgia is on the verge of adopting biosecurity rules modeled on the U.S. approach—but with some important differences. For instance, the same agency will regulate both biomedical and agricultural scientists; in the United States that job is split between the Centers for Disease Control and Prevention and the U.S. Department of Agriculture. "We're telling people that our model is often far more complicated than what they need," says a U.S. State Department official who advises other governments on biosecurity.

Building boom. Kazakhstan is the first of several nations getting new, secure laboratories to store and study dangerous pathogens. The facilities are courtesy of a U.S.-funded effort to reduce the bioterror threat in the former Soviet Union. Construction of the new Human Health Central Reference Lab and Repository in Almaty is set to begin in mid-2005, with Uzbekistan and Georgia next on the list. Meanwhile, talks are under way on long-term strategies for consolidating the 500 or more culture collections around the world that stock dangerous pathogens, with a goal of fewer, more secure facilities.

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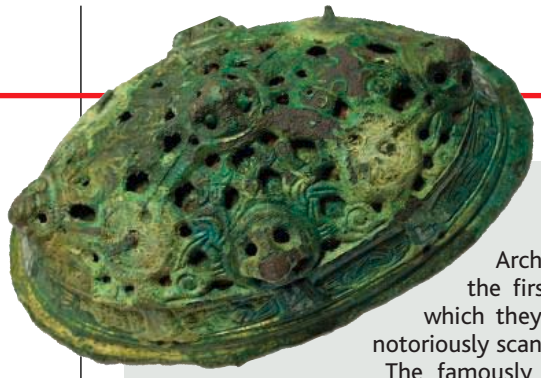


Viking brooch.

Viking Burial Site

Archaeologists are thrilled by the discovery of the first Viking graveyard uncovered in England, which they hope will shed light on a period with a notoriously scant record.

The famously fierce Vikings of Scandinavia surged up English shores in the 8th century and dominated until the Norman conquest in 1066, leaving a lasting legacy in terms of genes, language, and culture. But unlike their predecessors, the Romans, the Vikings rarely built permanent remains such as stone roads or buildings.

The find was made in March by an amateur, Peter Adams, wielding a metal detector on farmland in Cumbria, northwestern England. Archaeologists uncovered six graves with Viking objects including weapons, spurs, and jewelry that seem to date to the early 10th century. Little of the skeletons remain due to the acidic soil, but the objects suggest that the graves contained four men and two women. "To find just one grave is great," says excavation director Alan Lupton of the firm Oxford Archaeology North, who directed the 8-week excavation. "To find six is mind-blowing." University of Oxford archaeologist David Griffiths says there are Viking grave sites in Scotland and Ireland, but they were excavated in the 19th century under less than scientific conditions.

The graves show features of both pagan and Christian burial practices that could yield information about how Vikings made the transition from paganism to Christianity, says Lupton.

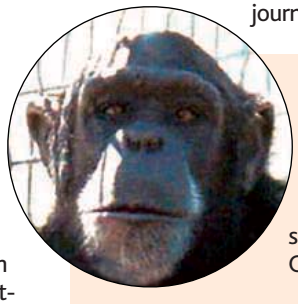
Defying Darwin

Promoters of intelligent design, the "scientific" wing of creationism, are gloating over a tactical victory this summer: the appearance of a critique of Darwinian evolution in a peer-reviewed biology journal. But the current editors say the journal shouldn't have published it.

The piece by Stephen Meyer, director of the Discovery Institute's Center for Science and Culture in Seattle, Washington, appeared last month in the *Proceedings of the Biological Society of Washington*, a small journal published by the Smithsonian Institution's Museum of Natural History. The article was accepted by then-editor Richard Sternberg, who does systematics research at the National Institutes of Health's GenBank. Sternberg is one of the signatories of "A Scientific Dissent from Darwinism," a statement circulated by the Discovery Institute.

The article has ruffled feathers at the journal. Its current editor, ornithologist Richard Banks, says Sternberg deviated from the journal's practice of assigning every submission to an associate editor. The society issued a statement calling the Meyer paper "a significant departure from the [journal's] nearly purely taxonomic content" and says the officers and editors "would have deemed this paper inappropriate for publication" if they'd known about it in advance.

Sternberg, who calls himself an advocate of "process structuralism," says the paper "was not outside the journal's scope" and was reviewed by three biologists. "Meyer set forth a reasoned view about the basis of taxa" which deserved airing despite being "politically incorrect," he says. The Discovery Institute has been making hay over the incident. "Darwinists try to thwart intellectual freedom," crows its Web site.



Ill-fated Ashley.

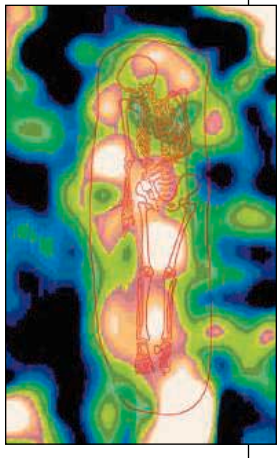
More Chimp Charges

The government facility in Alamogordo, New Mexico, that houses the former Coulston chimps faces new animal-welfare accusations—this time over the 2002 deaths of two sick chimps and the near-death of a third. On 7 September the Otero County district attorney filed animal-cruelty charges against Charles River Laboratories, which runs the facility for the National Institutes of Health (NIH), and the facility's director, veterinarian Rick Lee.

The animals are largely the offspring of those used in early space-flight experiments. Many were turned over in 1998 to the Coulston Foundation in Alamogordo. But in 2001, following accusations of negligence and cruelty, NIH put them in the care of Charles River (*Science*, 27 September 2002, p. 2191).

In Defense of Animals, a California-based group that has been critical of the facility, claims that care has still not improved. Based on reports from whistleblowers, the district attorney brought charges relating to the deaths or near-death of three severely sick or injured chimps, Rex, Ashley, and Topsy, who had been left overnight without medical care. Charles River issued a statement saying that they always provided "immediate and appropriate medical attention" to the animals in question. NIH said in a statement that the facility has passed inspection for proper accreditation but that the agency will "continue to review the issues."

Magnetic Memories



Skeletons often dissolve when buried in acidic soils. Now an archaeologist has shown that he can detect long-decayed remains by measuring the faint magnetic signal formed by soil microbes that enhance the magnetism of iron oxides from hemoglobin and other sources. This image, published in the current issue of *Archaeological Prospection* by archaeological geophysicist Neil T. Linford of English Heritage in Portsmouth, U.K., shows the magnetic signal from soil covering a roughly 1200-year-old Anglo-Saxon skeleton from Suffolk.

CREDITS (TOP TO BOTTOM): OXFORD ARCHAEOLOGY; N. T. LINFORD, *ARCHAEOL. PROSPECT.* 11, 1–13 (2004); IN DEFENSE OF ANIMALS

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Edited by Yudhijit Bhattacharjee

AWARDS

Rights warrior. Nguyen Dan Que, a Vietnamese endocrinologist currently under arrest in his home country, has received the 2004 Heinz R. Pagels Human Rights of Scientists Award from the New York Academy of Sciences. The 61-year-old doctor has spent 18 of the last 26 years in prison as a consequence of his efforts to promote human rights and democracy in Vietnam.

After graduating from Saigon Medical School in 1966, Nguyen practiced medicine in Europe on a scholarship from the World Health Organization but soon returned to Vietnam to provide free medical care for the poor. He became an outspoken critic of the government's health care policy and a champion of freedom of expression in the late 1970s.

Nguyen has been held incommunicado since his most recent arrest in March 2003. His brother, Quan Nguyen, an internist at Fairfax Hospital in Annandale, Virginia, says his brother does not know about the award but "would be very honored to receive it."

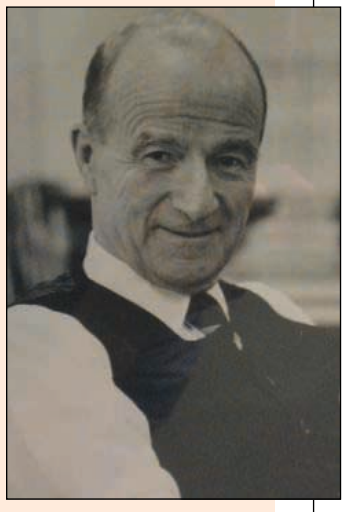
You go, girl. Women scientists received 60% of the National Science Foundation (NSF)–backed early career awards announced last week by the White House. That's a surprising outcome, given that the pool of young scientists from which NSF picks its winners is roughly 3-to-1 male. "The unmistakable message is that women have arrived," says NSF's acting director, Arden Bement.

Maybe at NSF, but apparently not at the other seven agencies that participated in the 2003 Presidential Early Career Awards for Scientists and Engineers (PECASE). NSF's contingent of 12 women (out of 20 winners) is twice the combined total of women in the rest of the PECASE class of 57. The National Institutes of Health's class of 2003, by comparison, contains 10 men and two women.

Herpetology honor. University of Wales biologist Simon Creer has won the Joseph B. Slowinski Award for Excellence in Snake Systematics from the Center for North American Herpetology. The award was created to honor Slowinski, a curator at the California Academy of Sciences in San Francisco, who died after being bitten by a venomous krait in Myanmar on 11 September 2001 (*Science*, 5 October 2001, p. 45). Thomas Wilcox of the University of Texas, Austin, won the first award last year.

DEATHS

Popularizer par excellence. Gerald Piel, a pioneer of modern science journalism and former publisher of *Scientific American*, died on 5 September in Queens, New York. He was 89.



A history major at Harvard University, Piel was science editor at *Life* in the 1940s before buying *Scientific American* with some friends in 1947. He introduced the idea of having scientists write popular accounts of their research, which helped boost its readership over the years. Piel remained an influential voice after stepping down as publisher in 1984, serving as president of AAAS (publisher of *Science*) in 1986 and writing books such as *The Age of Science: What Scientists Learned in the Twentieth Century* (2001).

"He was a landmark figure in journalistic letters, and he directly promoted the growth of science as much as any one person could," *Scientific American* noted in an obituary earlier this month.

JOBS

Continental vision. A French nuclear physicist–turned–policy wonk has been named the first director of the newly created European Space Policy Institute (ESPI) in Vienna, Austria. Serge Plattard takes up the reins of an institute, founded last November by the European Space Agency and the Austrian Space Agency (ASA), that will try to draw up a long-term framework for European space research. "We have lots of programs but no vision," he says.

Plattard, 57, anticipates having a staff of up to a dozen analysts and an annual budget of $1.8 million. "I know how to manage international and multidisciplinary teams," he says, "and how to attract nonstandard funding."

Plattard wants ESPI to be very independent and produce studies that can be instantly used by decision-makers. He's certainly up to the task, according to ASA's Michel Jakob, who says Plattard "can talk on the level of ministers and scientists. He can speak with everybody and be taken seriously."

Got any tips for this page? E-mail people@aaas.org

CREDITS (TOP TO BOTTOM): AAAS; QUAN NGUYEN; ESPI

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Hollywood, Climate Change, and the Public

IN HIS EDITORIAL "CLIMATE CHANGE AND climate science" (11 June, p. 1565), Donald Kennedy argues that although there is broad scientific consensus over the anthropogenic origin and growing seriousness of current climate change, public attention has instead focused on areas of scientific disagreement and on extreme but unlikely future scenarios. Against this background, there has been considerable debate about the potential impact on public opinion of the disaster film *The Day After Tomorrow*, in which global warming triggers a sudden shutdown of the Northern Atlantic Conveyor, bringing about a new ice age in a matter of days. Although some (*1*) argue that the film is likely to succeed in its makers' aim (*2*) of raising consciousness about climate change, others (*3*, *4*) suggest that by portraying dramatic but exceptionally unlikely events (*5*, *6*), it will instead reduce public understanding.

To shed light on this controversy, we collected data on 200 adults' levels of concern and knowledge about climate change, either on their way into (N = 95) or after seeing (N = 105) the film. Simple questionnaire surveys were conducted at four cinemas in southeastern England (Epsom, Putney, Sutton, and Wimbledon) in June and July 2004. Besides background socioeconomic data (age, sex, educational level, income, parental status, and environmental charity membership), questions focused on concern (measured as how much out of a hypothetical £1000 people wanted to give to climate mitigation versus four other good causes), on how many emission-reducing actions people already took or planned to take, and on knowledge of predicted temperature changes and their likely consequences (*7*, *8*). We assessed concern before asking questions that revealed that the survey focused on climate change. Here we examine the impact of the film by testing for differences in scores between arriving and departing filmgoers.

Watching *The Day After Tomorrow* did indeed raise levels of concern: Those questioned after seeing the film allocated almost 50% more of their hypothetical £1000 to climate mitigation than those interviewed on arrival [means ± SE of £180.13 ± £15.20 versus £122.25 ± £13.20; $t_{184} = -2.67$, $P < 0.01$ (*9*)]. Moreover, this exit-entry difference remained significant after controlling for the positive effect of parenthood (*10*). There was no difference in how many kinds of emission-reducing actions (such as using public transport or low-energy bulbs) that our exit and entry sample already undertook (1.71 ± 0.10 versus 1.80 ± 0.10; $t_{190} = 1.15$, NS), but the film also had no detectable effect on the number they planned to undertake in future (2.56 ± 0.11 versus 2.64 ± 0.11; $t_{191} = 0.71$, NS). There was no change, after seeing the film, in people's score for knowledge of likely 21st-century temperature change (2.60 ± 0.07 versus 2.62 ± 0.06; $t_{171} = 0.26$, NS), despite twice as many exiting filmgoers suggesting it will become colder. However, when asked which specific effects of climate change are predicted (*7*, *8*) for the UK by 2100, those who had seen *The Day After Tomorrow* had less realistic expectations than those interviewed beforehand (*11*).

Overall, the data thus suggest that seeing an entertaining if exaggerated illustration of the possible effects of climate change succeeds, in just 125 minutes, in raising public concern—but at the price of reducing public understanding. It would be interesting to see if these divergent effects hold in other countries, and which, if either, persists over time. More generally, our findings confirm that intense dramatizations have real potential to shift public opinion. However, the question remains whether such portrayals can be made more accurate (and thereby less confusing) without losing their popular appeal.

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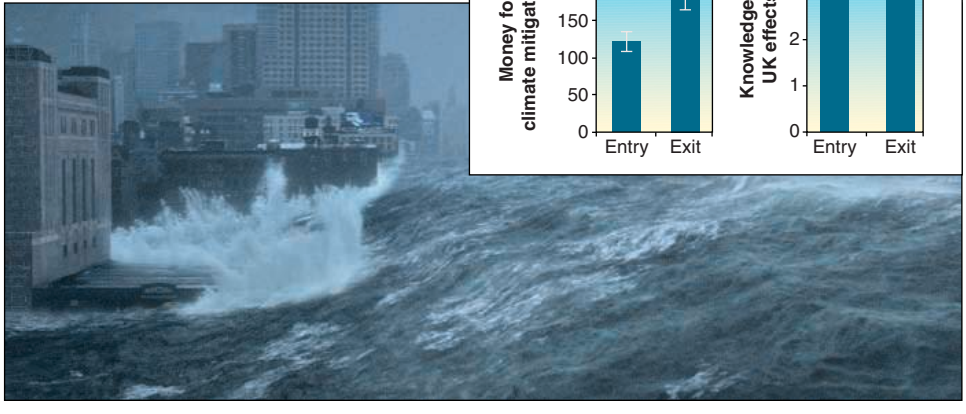

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Impact of watching *The Day After Tomorrow* on people's concern and knowledge about climate change. Concern is measured as how much out of a hypothetical £1000 people wanted to give to climate mitigation versus four other good causes (social or medical charities, overseas aid, or animal welfare). Knowledge is scored as how many out of six possible effects (increased winter flooding, house subsidence, late frosts, earlier flowering, lawn-mowing in winter, and the Thames freezing solid) respondents correctly identified (*8*) have or have not been predicted for the UK by 2100. The histograms show means ± SE. The photo shows a scene from *The Day After Tomorrow*, in which a tidal wave hits New York City.

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9. All t tests used arc-sine square-root-transformed scores, expressed as percentages of the maximum possible.
10. A minimal Generalized Linear Model (GLM) with quasi-binomial error structure gave $F_{2,197} = 7.19$, $P = 0.001$, with $P < 0.05$ for both terms; no significant interactions.
11. Mean scores (out of 6): 3.06 ± 0.13 versus 3.48 ± 0.13; $t_{179} = 2.57$, $P = 0.01$; with the effect remaining significant in a GLM controlling for sex and the positive effects of income and charity membership: $F_{4,195} = 8.43$, $P < 0.001$, with $P < 0.05$ for all four terms; no significant interactions.
12. Readers interested in replicating this survey should contact the corresponding author for a copy.

Evidence for Taming of Cats

THE RECENT BREVIA CONCERNING EVIDENCE OF early taming of cats ("Early taming of the cat in Cyprus," J.-D. Vigne *et al.*, 9 Apr., p. 259) contains some statements that I feel should be scientifically tempered. Vigne *et al.* state "The joint burial could also imply a strong association between two individuals, a human and a cat… The Cyprus burial thus likely represents early evidence for the taming of cats."

The Cyprus find is at best evidence of a relationship between an admired human being and a possibly commensal species of wild animal, *Felis sylvestris*. Students of feline systematics have long identified the African wild cat (*F. silvestris*) as the sister group to the domestic cat (*1*–*3*). However, in the absence of any artifacts of domestication at the Cyprus site (collar, clothing, cage, evidence of castration of male, jewelry on the cat, and so forth), the felid in question must be considered a wild commensal species.

I think it is fair to say that the first status or stage of this process was that of a commensal variety of the wild cat. It is further reasonable to assume that the wild cat was welcomed in the vicinity of early humans, as it helped control a less acceptable commensal animal, the rodent. The transition from the African wild cat to a truly domesticated feline would have involved living and breeding as a tame member of a human dwelling. As a paleontologist and a veterinarian, I suggest we seek more hard evidence before using the term "tame" or "domesticated."

TOM ROTHWELL

Division of Paleontology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA.

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Response

WE THANK ROTHWELL FOR HIS REMARKS. IT IS important to remember that, as in paleontology, archaeological results can never be considered as certainties, but only as probabilities. However, we continue to suggest that "[t]he Cyprus burial… likely represents early evidence for the taming of cats" and not merely a manifestation of a commensal status of a wild species.

The cat is one of the seven species of large mammals, among the several tens of wild species once present in Western Asia, that were introduced voluntarily onto the island of Cyprus at the very beginning of the Neolithic, across an 80-km-wide sea channel. Five of the other introduced species (dog, sheep, goat, pig, and cattle) subsequently became the main domesticated animals of the Middle East. The probability that the cat could have travelled across the sea on its own seems to us to be extremely small.

During the 9th and the beginning of the 8th millennia B.C.), the house mouse and the fox (*1*) share with the cat the peculiarity of having been both commensal and introduced onto Cyprus. Like the cat, the fox must have been voluntarily introduced by humans; however, unlike the cat, the fox did not become a domestic species in the following centuries. Although much more frequently recovered in the Cyprus Neolithic sites than those of the cat, its bone remains have never been found in a burial or in any symbolic situation, but only as food refuse.

As we argue in our Brevia, "[t]he burial of a complete cat… emphasizes the animal as an individual." In this Middle East complex of cultures of the Pre-Pottery neolithic, when wild animal species appear in any kind of cultic contexts, they are symbolically represented by heads, horncores, antlers, jaws, scapula, and so forth, very rarely as a complete individual (*2*, *3*); only human beings and some domestic animal with special status such as dogs in the Natoufian culture or some sheep in the Khirokitia culture (*4*) are buried as complete bodies.

Rothwell seems to base his arguments on an old concept of the genus *Felis*. According to the most recent phylogenetic works (*5*), what he calls "the African wild cat" (i.e., the *lybica* subspecies of *F. silvestris*) also includes the wild cats of the Near East. Consequently, the proximity between the domestic cat and the African wild cat does not mean that the ancestor of the domestic cat is lived in Africa.

Furthermore, the majority of modern domestic animals (and probably in Neolithic times) have no "collar, clothing, cage, … jewelry" and are not castrated. This kind of evidence is of little use in detecting early domestication. Among the few criteria that are validated by most palaeoanthropologists (*6*) are the occurrence of a taxon previously unrecorded in a region and incorporation into a society's symbolic system. These are the two main arguments that we use for suggesting that the Cyprus cat was tamed.

Finally, Rothwell is correct when he writes "The transition… to a truly domesticated feline would have involved living and breeding as a tame member of a human dwelling." But it seems to us and to numerous other archaeologists that the purposeful introduction of the cat onto the island at a time when seafaring was probably still in existence and its exceptional presence in the environment of the burial suggest that at least some cats did live in

such conditions in Cyprus during the preceramic Neolithic period.

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Figuring Out What Works in Education

IN HIS ARTICLE "MEAGER EVALUATIONS MAKE IT hard to find out what works," J. Mervis writes about the difficulties of assessing education research (News of the Week, 11 June, p. 1583). The only way to establish a curriculum's effectiveness is through randomized, control trials similar to those used in clinical research. The idea of controlled trials in evaluation is certainly not new, and I learned about them in the 1970s while studying for a Ph.D. in education. Unfortunately, interest in educational program evaluation has remained theoretical in schools. In fact, in frustration, I left education studies and moved to academic medicine, where effectiveness research and systematic literature reviews to find best practices are the norm (*1*). I take issue with the idea that controlled studies are difficult to do because "[i]t's hard to exclude people from a program that they think is working." Controlled studies are based on rational inclusion and exclusion criteria and aim to demonstrate under which circumstances and for whom programs work best (if they work at all). Also, although it is true that measuring human behavior is "hard to quantify," that does not mean we cannot do it reasonably well. In academic medicine, we have found ways to measure concepts like satisfaction, decision-making, and quality of life. Surely, with effort, educational evaluators can identify valid measures of educational outcomes.

One possible explanation for the poor studies we find in education is that evaluations cost as much as program development. The best ones are conducted by multidisciplinary teams of educational researchers and experts in design, measurement, and data analysis. Educational programs are always in a cash crisis. Yet the consequences of implementing programs of unknown quality are likely to be even more costly in terms of the educational opportunity they waste.

ARLENE FINK

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Reference

1. See, for example, B. Stein *et al.*, *JAMA* **290**, 603 (2003).

CORRECTIONS AND CLARIFICATIONS

News of the Week: "Patient advocate named co-inventor on patent for the PXE disease gene" by E. Marshall (27 Aug., p. 1226). The article incorrectly stated that Charles Boyd's group at the University of Hawaii was first in a four-way race to report the gene 4 years ago. Although the four groups published within a few weeks, and Boyd's group holds the patent, the first report in print was by Jouni Uitto and colleagues at the Thomas Jefferson University in Philadelphia.

Random Samples: "Side effect" (6 Aug., p. 775). The item should have noted that University of Pittsburgh geneticist Robert Ferrell resigned as chair of his department because he is undergoing treatment for recurring non-Hodgkins lymphoma.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "The Early Evolution of the Tetrapod Humerus"

P. E. Ahlberg

Shubin *et al.* (Reports, 2 April 2004, p. 90) claimed that specimen GSM 104536 from Scat Craig, Scotland—described as the earliest known tetrapod humerus—entirely lacks tetrapod characteristics. This unsupported assertion contradicts the published descriptions and comparative analyses of the specimen, and thus misrepresents this potentially important fossil.

Full text at www.sciencemag.org/cgi/content/full/305/5691/1715c

RESPONSE TO COMMENT ON "The Early Evolution of the Tetrapod Humerus"

Michael I. Coates, Neil H. Shubin, Edward B. Daeschler

Virtually all of the features used to associate GSM 104536 with tetrapod humeri are difficult to assess either because of preservation or differences between tetrapods and their fish relatives. This leaves two alternatives: Either the bone is not a tetrapod humerus or it represents forms far beyond the known range of early humeral diversity. In the absence of more complete material, this bone remains enigmatic.

Full text at www.sciencemag.org/cgi/content/full/305/5691/1715d

ENVIRONMENT

How Best to Face the Coming Storm

Partha Dasgupta

Red Sky at Morning
America and the Crisis of the Global Environment
James Gustave Speth
Yale University Press, New Haven, CT, 2004. 317 pp. $24, £15.99, €25.60. ISBN 0-300-10232-1.

Twentieth century economics has in large measure been detached from the environmental sciences. Judging by the profession's writings, we economists see nature at best as a backdrop from which resources can be considered in isolation; we also imagine most natural processes to be linear. Macroeconomic forecasts routinely exclude environmental resources. Accounting for nature, if it comes into the calculus at all, is an afterthought to the real business of "doing economics."

One can argue that this practice has given rise to a puzzling cultural phenomenon: One group of researchers (usually natural scientists) sees in humanity's current use of nature's services symptoms of a deep malaise, even while another group (usually economists) documents the fact that people today are on average better off in many ways than they had ever been and wonders why the gloom.

Underlying the intellectual tensions are the conflicting intuitions that have arisen from different empirical perspectives on whether the character of contemporary economic development, both in the poor world and in industrialized countries, is sustainable. On the one hand, if we look at specific resources and services (such as fresh water, a wide variety of ecosystem services, and the atmosphere as a carbon sink), there is convincing evidence offered by earth scientists that continued growth in the rates at which they are utilized is unsustainable. On the other hand, if we study historical trends in the prices of marketed resources (minerals and ores, for example) or the recorded increases in the conventionally measured indices of economic progress [such as per capita gross national product (GNP) and the United Nations' human development index] in large numbers of countries, environmental and resource scarcities would not appear to have bitten yet.

Over the past few years, there has arisen a small but professional literature that reconciles the conflicting intuitions. It does so by establishing that, roughly speaking, sustainable development involves the maintenance of wealth, where the required measure of wealth includes not only manufactured capital (buildings and machinery) and human capital (knowledge, skills, and health) but also natural capital (ecosystems). This literature has identified the many circumstances in which a nation's GNP per capita would increase over a period of time and its human development index improve, even while its wealth per capita declines. Evidence suggests that South Asian countries have experienced this pattern of economic change during the past three decades, while sub-Saharan Africa has enjoyed an improving human development index despite suffering declines in GNP per capita and wealth per capita (*1*, *2*). In broad terms, the circumstances of such experience involve growing markets in certain classes of goods and services (such as petroleum products and transportation), concomitant with an absence of markets and collective policies for environmental goods and services. Moreover, global environmental problems often percolate down to create additional stresses on the local resource bases of the world's poorest people. The overall point is that when a commodity is unpriced, there is no incentive to economize on its use.

In *Red Sky at Morning*, James Gustave Speth takes us on a guided tour of those global (and largely interconnected) environmental problems that are recognized by experts today to be in the most urgent need of attention. Speth, the distinguished dean of the School of Forestry and Environmental Studies at Yale University, marshals facts and figures in measured tones. Refreshingly, he makes use of findings from both the social and earth sciences to sketch some of the processes that characterize human-nature interactions. This permits him to explore fundamental explanations underlying environmental degradation. For example, he recognizes that the more than fourfold increase in human numbers during the 20th century has been a proximate cause of environmental degradation, so he identifies several societal features responsible for that increase. There is no hysteria here, nor any attempt at cowering the reader with statistics: the style is that of a kindly uncle rebuking his many nieces and nephews for the errors of their ways.

As would be expected of someone who previously administered the United Nations Development Programme, Speth is engaged not only in reporting global environmental degradation but also in identifying institutional reforms that are necessary if we are to successfully address the problem. In a far-reaching trio of chapters toward the book's end, he argues for the establishment of a new international organization that would act on behalf of the environment in much the way the World Trade Organization acts on behalf of a rational trading order. It was only when I came to those concluding chapters that I realized that the earlier sections of the book were intended as a sort of intellectual prelude: Speth's proposal for a new organization appears as a logical necessity, borne out of humanity's experience in dealing with nature. *Red Sky at Morning* is a most readable and altogether fine book. I can only hope it becomes a most influential one.

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ANTHROPOLOGY

Culture and Commerce in a Seafood Bazaar

Stephen Gudeman

Bronislaw Malinowski launched modern ethnography in the early 20th century with his study of gift exchange in the Trobriand Islands of the South Pacific. He showed how reciprocity—the give and take of things and services—provided the glue for social life and reflected the many strands of Trobriand culture. Late in life, Malinowski turned to the study of a market in Mexico, as if it might be the counterpart to reciprocity in making the economy and refracting parts of

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culture. Since Malinowski, ethnographies and studies of reciprocity have flourished, but the world has changed. Can today's anthropologists help us understand contemporary economies and markets? Given the impact of postmodernist theory, can they even claim that ethnographies represent what a people do and think?

Tsukiji, Thomas Bestor's study of the Tokyo market that is the world's largest marketplace for fresh and frozen seafood, convincingly returns us to the ethnographic tradition. At the Tsukiji market in 1966, nearly 6 billion dollars of seafood changed hands, a volume of trade that dwarfs that of all other fish markets. Bestor, a professor of anthropology at Harvard University, began his research in 1989 and continued visiting the market for ten years. With his intimate knowledge of Tsukiji, he provides the reader with an ethnography filled with descriptions, "facts," stories, verbal pictures, and a feeling for the sounds and tastes of the place and culture.

The study revolves about the theme that Tsukiji trade is embedded in the relationships, beliefs, and values of Japanese life. The idea of embeddedness is not new, but Bestor uses it to advantage and displays its power. For example, from the perspective of an economist, Tsukiji is a "spot market" in which goods are exchanged between anonymous actors for short-term advantage and profit maximization. In contrast, Bestor demonstrates how Japanese social patterns enable and constrain the competitive trade. For Bestor, the notion of embeddedness has a very broad reach.

Tsukiji:
The Fish Market at the Center of the World
Theodore C. Bestor

University of California Press, Berkeley, 2004. 440 pp. $60, £39.95. ISBN 0-520-22023-4. Paper, $24.95, £16.95. ISBN 0-520-22024-2. California Studies in Food and Culture.

The Tokyo fish market probably dates to the 1500s, although the current marketplace was constructed only in the 1920s. Bestor shows how its history, changing customs, and physical layout all affect price-setting. In addition, the seafood sold at Tsukiji comes from local fishermen and from all parts of the world, so pricing reflects global trends. Because demand is local to Tokyo, Bestor also explains how Japanese tastes and habits drive the market yet are influenced by recent changes in domestic arrangements and food preparation as well as by global fashions.

At daily auctions, licensed buyers, who supply restaurants and other outlets in Tokyo, bid through a series of hand gestures. Seven auction houses lie at the center of the market. Though most specialize in certain products, they compete with one another and with other markets. All seven have semi-permanent relationships upstream with suppliers and downstream with purchasers. These bonds modulate market swings through the transmission of information and the lowering of transaction costs that would otherwise be incurred. In addition to these semi-permanent exchange relationships and government licensing and regulations, the market is permeated or institutionalized by *keiretsu*, which are vertically integrated combines that link fisheries, trading companies, and supermarket chains. The combines stabilize the market and limit competition. Yet market embedding extends further. For example, small retail firms that purchase fish for sale and keep stalls at the marketplace are often organized around family ties. Their risk is modulated by the Tsukiji practice of reassigning the stalls every four to five years by a lottery and by formal and informal regulations that dampen competition.

A range of readers, from regional specialists to tourists, will be interested in this well-rounded study that displays the merits and skills of ethnography; Bestor even provides an appendix telling visitors how to visit and what to see in this fascinating place. In some respects, however, the work does not resolve the issue on which it is based: embeddedness. If markets give vent to rational choice, self-interest, sturdy individualism, and profit making, how is such behavior reconciled with the many ways in which they are circumscribed by cultural values and social relationships? Disregarding differences between the approach of anthropologists and sociologists on the one side and formal economists on the other, is there an inherent tension in the economy itself? How do people experience, feel, and voice this tension between self-interested maximization and mutuality? Some readers might wish to know whether local voices express this tension, to learn more about its potentially dynamic effects on the market, and to be exposed to the "incompletion" of economic life in Tsukiji. But Bestor provides one of the finest ethnographic studies that we have of a modern market, and he can address these questions in his next dispatch. If you visit Japan, take this book for its insights into the culture, a Tokyo neighborhood, and the local diet.

Simpler exchange. Until the 1923 earthquake, Tokyo's fish trade was concentrated at Nihonbashi, which is depicted in "Clearing After Snow, Nihonbashi" from Hiroshige's 1856 series *One Hundred Famous Views of Edo*.

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BROWSINGS

True Warnings and False Alarms. Evaluating Fears about the Health Risks of Technology, 1948–1971. *Alan Mazur.* **Resources for the Future, Washington, DC, 2004. 199 pp. $50. ISBN 1-891853-55-4. Paper, $18.95. ISBN 1-891853-56-2.**

How seriously should we take claims of a newly discovered threat to public health? To help us judge, Mazur analyzes 31 claims from the 1950s and 1960s about threats posed by a variety of technologies, including radioactive wastes, DDT, fluoridation, mercury pollution, oral contraceptives, and medical x-rays. In this retrospective risk analysis, he reviews the sources and circumstances of the claims, looking for differences between the 18 that are accepted as valid and the 13 false alarms. He finds that warnings were more likely to be true if they were first raised in reports of research by recognized scientific organizations rather than claims by advocacy groups or government agencies. For warnings from the latter sources, those that appeared in isolation (rather than an already charged atmosphere) were more accurate. However, there seemed to be no relation between validity and the extent to which news coverage had hyped the claim.

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GENETICS

Ethical Aspects of ES Cell–Derived Gametes

Giuseppe Testa* and John Harris

Three laboratories recently reported derivation of gamete-like cells from mouse embryonic stem (ES) cells (*1*–*4*). Much progress must be made to reach the goal of deriving bona fide germ cells from ES cells; the chief test will be their ability to sustain normal fertilization and development. However, these cells appear to share, to some degree, fundamental features of gametes, namely, a haploid genome and erasure of imprints (i.e., the molecular marks that distinguish alleles according to their origin and are essential for normal development).

The most immediate application of ES-derived oocytes will likely be in somatic cell nuclear transfer (SCNT) (*5*) for research purposes. Clearly, many challenges remain before such gametes could be used in human reproduction. Precisely because the technology is not yet within immediate reach, we believe it is timely to start developing a bioethical and legal discourse.

The possibility of deriving gametes from human ES cells could allow infertile individuals to have genetic offspring, unless the defect also impaired in vitro generation of gametes. Infertility affects roughly 2.1 million couples in the United States alone (*6*). A somatic nucleus from the infertile member of the couple would be transplanted into an enucleated oocyte. ES cells would be derived from the blastocyst stage of this cloned embryo and differentiated into gametes, which would be used for in vitro fertilization (IVF) with the naturally generated gamete from the fertile partner. Although the procedure involves SCNT, this would not amount to reproductive cloning, because the offspring would have, as in normal reproduction and IVF, the genomic contribution of both parents. Simply, whereas the haploid genome from the fertile member of the couple will have been reprogrammed ("made fit for fertilization") through the normal process of gametogenesis, the infertile member will have achieved the same result with an "assisted reprogramming effort" mediated through the resetting activity of a donor oocyte. We suggest that from an ethical and legal perspective, this procedure is most appropriately framed as a therapeutic intervention to treat infertility. It replaces in vitro the physiologic function normally responsible for reprogramming the germline genome, analogously to the well-established medical technologies that replace other deficient bodily functions.

If the technique approaches the same level of safety and cost as those of current IVF protocols, it is likely that the majority of infertile individuals and/or couples would want to benefit from it. However, this does not imply any preeminence of genetic over social parenthood. Although our society values social parenthood highly, it still actively encourages those who can to have genetic offspring. Provided that the technology would be widely and justly accessible, this could further democratize human reproduction.

As with any medical procedure, safety would be a crucial parameter. Faulty imprinting causes severe abnormalities, and careful experiments in animal models will have to assess the imprinting status of ES-derived gametes (ESDG). Another potential problem could result from the accumulation of genetic damage that occurs in a somatic cell. However, ethical arguments based generally on DNA integrity would not be tenable if it were to be shown that oocytes or sperm from older individuals have amounts of damage similar to those of somatic lineages used for ESDG generation. As with IVF, evaluation of safety always entails a comparison with the safety of natural or other accepted processes rather than of ideal absolutes. The identification of the normal standard for any biological system is always a complex settlement between our incomplete knowledge and the risks we are prepared to take as individuals and as a society.

ESD oocytes have been derived from male (XY) and female (XX) mouse cells (*1*). If the process could be successfully completed with human ES cells, i.e., derivation of a mature oocyte from a male ES cell line, this would have important social implications. Two men could potentially have a child to which both parents contribute their genomes, one through the natural process of spermatogenesis, the other through the assisted process of genome reprogramming down the female germline. A woman would need to carry this embryo to term. The possibility of an all-male or all-female couple's (*7*) being able to have a child sharing the genetic make-up of both parents in virtually the same way as for heterosexual couples is thought-provoking and can be used as a lens through which to discern our attitudes toward parenting and family, as well as our notions of what is "natural."

Same-sex couples can use assisted reproductive technologies (ART) in the United Kingdom to have children to whom they are at least in part genetically related and are able to adopt children in several countries. There have been extensive analyses of the success of same-sex couples as parents in the context of ART and adoption (*8*). As with most forceful rejections of social changes that violate presumed natural laws (*9*, *10*), so for ESDG-based technology, most objections will likely arise from the idea that the resulting child will somehow be harmed by the nature, novelty, or "strangeness" of the process itself. This is analogous to the early days of IVF, when it was suggested that "test tube" babies might not have a soul (*11*). In our view, not only does a child born as a result of ART or ESDG obviously have the same moral status as a child born by natural reproduction, but would also have no special advantages or disadvantages. Natural reproduction may be more enjoyable for humans than the in vitro approaches, and that would certainly be a good reason to prefer it. However, there is no a priori reason to prefer the natural, for the natural per se is morally neutral. The whole practice of medicine is a comprehensive attempt to frustrate the course of nature. If we always preferred the natural as a matter of principle, we would have to abjure medicine altogether.

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12. The authors acknowledge support from the Branco Weiss Fellowship Society-in-Science and a project grant from the European Commission.

NEUROSCIENCE

Signposts to the Essence of Language

Michael Siegal

In one of the most dramatic incidents of the French Revolution, the Abbé Sicard, director of the school for the deaf in Paris, failed to take an oath of civil allegiance. As described by Lane (*1*), he was imprisoned and sentenced to die. However, Sicard, who was devoted to establishing communication through sign language, was rescued through the pleas of his deaf students. They petitioned the National Assembly for his release, testifying that without him they would be like animals.

Deaf people have fiercely resisted century-old attempts to prevent them from using their own sign language for communication (*2*). They argue that sign language is equivalent to spoken language and that users of a sign language should be accorded the same rights as users of a spoken language. The origin and core properties of sign language, however, remain to be elucidated. On page 1779 of this issue, Senghas *et al.* (*3*) address this lack of information in their landmark study of three cohorts of deaf Nicaraguan signers. Their research is based on the passion for sign language of several generations of deaf children attending a special education program set up in 1977 in the Nicaraguan capital, Managua. In the 30 years since the program opened, the children have created a completely new language—Nicaraguan Sign Language (NSL)—that has continued to expand and mature and has been passed on from one group of children to the next (see the photographs). There are about 800 deaf NSL signers, ranging in age from 4 to 45 years. NSL is one of hundreds of distinctive sign languages in existence around the world (see the figure). The creation of NSL has allowed unique insights into the essence of language—both sign and spoken.

Segmentation and sequencing are considered vital core properties of all languages. In their investigation, Senghas *et al.* explicitly analyzed the segmentation and sequencing in NSL of elements such as motion. The authors did this by showing animated cartoon videos to three cohorts of NSL signers of different ages and to a sample of hearing Spanish-speaking Nicaraguans. In one of these videos, a cat swallows a bowling ball and wobbles (manner of movement) as it descends (path of movement) down a steep road. The first cohort of Nicaraguan signers, who were the initial builders of NSL, represented manner and path information simultaneously in a single movement of the hand, much as the Spanish speakers did in the gestures that accompanied their speech. In contrast, the second and third cohorts of NSL signers overwhelmingly produced sequential hand movements involving strings of segmented manner-only and path-only elements. Such segmentation and sequence elements can be embedded within other signs (phrases) to build a hierarchical organization of information that forms an elaborate communication system. Intriguingly, NSL has evolved from a system of nonlinguistic gestures into a full sign language with its own grammar that continues to expand and mature. Consequently, because they have learned the language most recently, the youngest children in the NSL community are the most fluent signers.

Senghas *et al.* observe that segmenting and sequencing depend on combining elements of language within a hierarchical structure that permits the generation of an infinite number of messages. The mechanisms through which segmentation and sequencing are achieved in NSL challenge the position that language evolves through

Fluency among the youngest. Nicaraguan children communicate through a sign language (NSL) that they developed over a 30-year period. The opening of an education program in 1977 in Managua (the capital of Nicaragua) brought together a community of deaf children for the first time in that country. The children developed their own sign language, which evolved from nonlinguistic gestures to a full grammatical language that continues to mature. The youngest children in the NSL community are the most fluent signers, having learned the language most recently.

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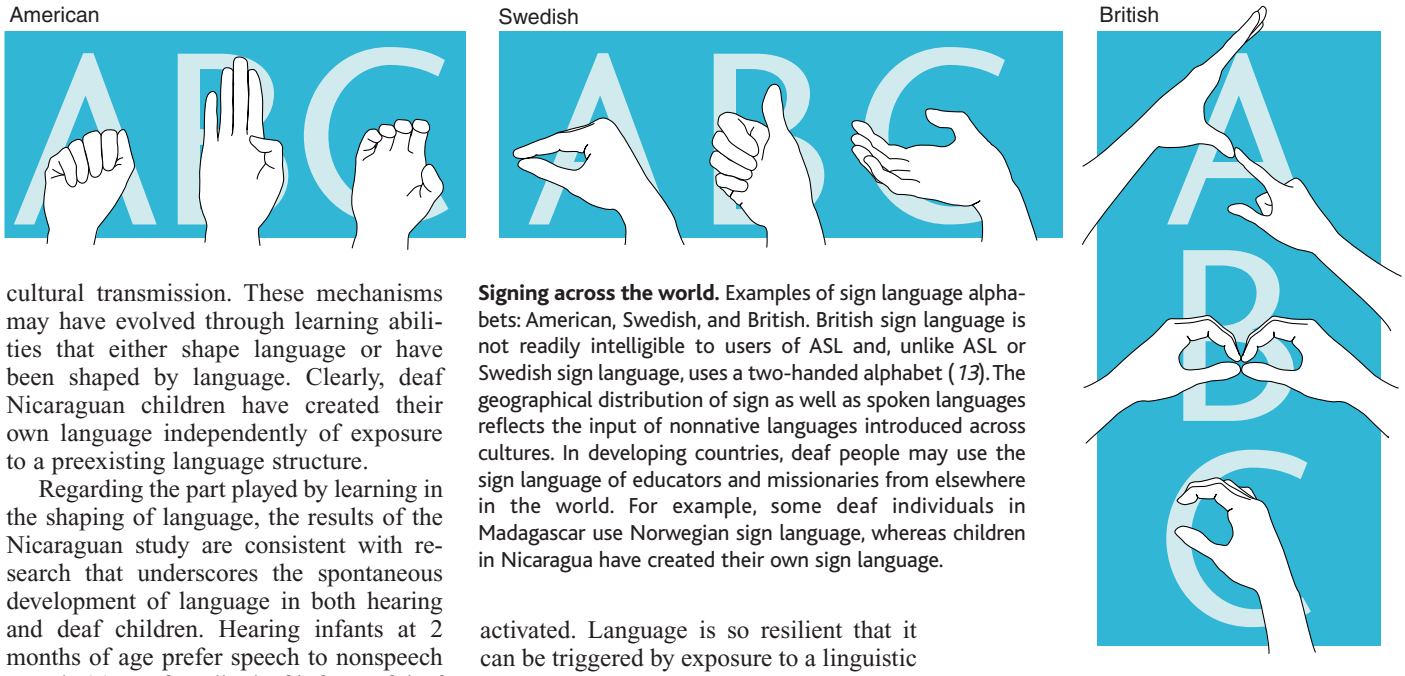

Signing across the world. Examples of sign language alphabets: American, Swedish, and British. British sign language is not readily intelligible to users of ASL and, unlike ASL or Swedish sign language, uses a two-handed alphabet (*13*). The geographical distribution of sign as well as spoken languages reflects the input of nonnative languages introduced across cultures. In developing countries, deaf people may use the sign language of educators and missionaries from elsewhere in the world. For example, some deaf individuals in Madagascar use Norwegian sign language, whereas children in Nicaragua have created their own sign language.

cultural transmission. These mechanisms may have evolved through learning abilities that either shape language or have been shaped by language. Clearly, deaf Nicaraguan children have created their own language independently of exposure to a preexisting language structure.

Regarding the part played by learning in the shaping of language, the results of the Nicaraguan study are consistent with research that underscores the spontaneous development of language in both hearing and deaf children. Hearing infants at 2 months of age prefer speech to nonspeech sounds (*4*). Profoundly deaf infants of deaf parents display manual babbling using a reduced set of the phonetic units in American Sign Language (ASL) in a manner analogous to the vocal babbling of hearing infants exposed to a spoken language (*5*). In the first few years of development, virtually all children—whether hearing children exposed to a spoken language or deaf children exposed to the sign language of their deaf parents—acquire the grammar of their native language. The structure of spontaneous gestural communication ("home signing") of American deaf children resembles more closely that of deaf children in Taiwan than that of their own hearing mothers (*6*). Language involves "language-making" skills—segmenting words into morphemes and sentences into words, setting up a system of contrasts in morphology, and constructing syntactic structures—that do not require a language model to be activated. Language is so resilient that it can be triggered by exposure to a linguistic input that is highly limited and fragmented—an indication of the fundamental inateness of grammar (*7*, *8*).

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Early language exposure shapes linguistic ability, in that those who become deaf after having acquired spoken English appear to be more proficient in learning ASL than those born profoundly deaf with little linguistic experience before exposure to ASL at school. In contrast, deaf people who are exposed early to ASL are able to learn spoken English better than those who have been exposed late (*9*). But do such language-shaped learning mechanisms stop there? Can they be extended to allow or facilitate the acquisition of, for example, mathematics or propositions about the beliefs held by the minds of others? One question to be resolved is whether language entails a learning mechanism that instantiates mathematical reasoning, given that language and mathematics share similarities in syntactic structure (*10*). Another question is whether the syntactic structure of language allows us to entertain propositions—for example, "John thought that Mary knew the cookies were in the cupboard"—that permit insight into the false beliefs of others, or whether it is early access to conversations that alert children to the notion that beliefs can differ from reality (*11*). Also, it is not clear whether the innate structure of language allows processing of causal and counterfactual reasoning.

In this light, language can be regarded as mandatory to human development with widespread, although as yet undetermined, implications for the nature of cognition. Without access to language, our communication would rely on iconic representations that are within the grasp of nonhuman primates and even pigeons (*12*). The Nicaraguan research highlights segmenting and sequencing as core linguistic properties that develop innately and not as a result of cultural transmission. Such inateness confers humanity on both deaf and hearing people through language creation and immersion.

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CELL BIOLOGY

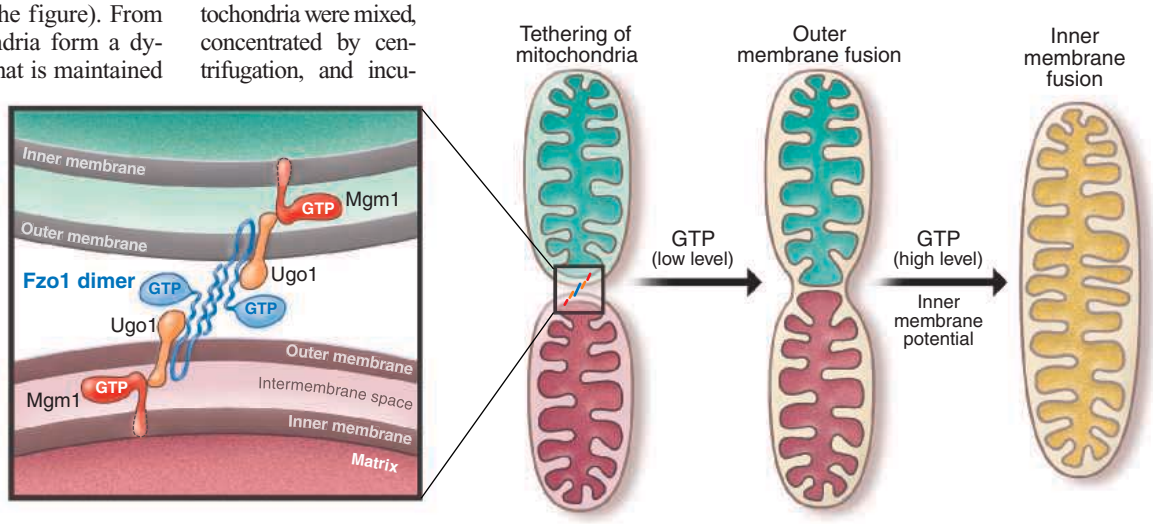
Double Membrane Fusion

Nikolaus Pfanner, Nils Wiedemann, Chris Meisinger

Mitochondria are cytoplasmic organelles that are crucial players in numerous cellular processes. These include bioenergetics, metabolism of amino acids, lipids, and iron, as well as programmed cell death (apoptosis), differentiation, and aging (*1*–*4*). Mitochondria comprise two membranes, the outer membrane and the folded inner membrane, and two aqueous compartments, the intermembrane space and the matrix (see the figure). From yeast to humans, mitochondria form a dynamic network in the cell that is maintained by balancing mitochondrial fusion and fission. The dynamic morphology of mitochondria is critical for the inheritance of mitochondrial DNA, for the transmission of energy, and for cellular differentiation, such as spermatogenesis. During apoptosis, enhanced mitochondrial fission and diminished mitochondrial fusion lead to mitochondrial fragmentation, potentiating the cell death response. Defects in mitochondrial fusion have been linked to neurodegenerative diseases like dominant optic atrophy and Charcot-Marie-Tooth type 2A disease, an inherited peripheral neuropathy. In vivo studies in yeast, *Drosophila*, and mammalian cells have led to the identification of many proteins that are involved directly or indirectly in the maintenance of mitochondrial morphology. However, lack of an in vitro system for studying mitochondrial fission and fusion has hampered analysis at the molecular level. This is now remedied by Meeusen *et al.* (*5*) reporting on page 1747 of this issue. They describe an elegant in vitro assay that follows the fusion of isolated mitochondria, providing important insights into the ordered events of outer- and inner-membrane fusion.

Mitochondrial fusion has been considered a complicated process that requires essential cytosolic elements. Yet the assay established by Meeusen *et al.* is remarkably straightforward and does not require the addition of cytosolic factors. This implies that isolated mitochondria contain all of the essential ingredients required for fusion. Meeusen *et al.* used mitochondria labeled with matrix-targeted molecules: either green fluorescent protein (GFP) or red fluorescent protein (dsRED) (see the figure). Differently colored mitochondria were mixed, concentrated by centrifugation, and incubated in the presence of guanosine triphosphate (GTP) and energy substrates to generate an inner-membrane electrical potential. Mitochondrial fusion could be monitored directly through mixing of GFP and dsRED such that the matrix of fused mitochondria contained both fluorescently labeled proteins (see the figure). The centrifugation step is a crucial element of the assay because it brings mitochondria into close proximity. In vivo, this step is probably accomplished by the cellular cytoskeleton (*5*, *6*).

With their assay, Meeusen *et al.* dissected mitochondrial fusion into sequential outer- and inner-membrane fusion events. Outer-membrane fusion required low levels of GTP and a mitochondrial proton gradient. Inner-membrane fusion depended on the hydrolysis of large amounts of GTP and the presence of an inner-membrane electrical potential. The GTP dependence of mitochondrial fusion concurs with previous in vivo studies that revealed a requirement for two large dynamin-related guanosine triphosphatases (GTPases): Fzo1 (*fuzzy onions* or mitofusin) of the outer membrane, and Mgm1 (mitochondrial genome maintenance) of the intermembrane space (*1*, *2*, *4*). In yeast cells, Fzo1 and Mgm1 are linked together in a complex by the outer-membrane protein Ugo1 (derived from the Japanese word for fusion) (*7*, *8*). Homologs of the fusion machineries that operate in the transport of vesicles (such as SNARE proteins) (*9*) do not seem to be involved in mitochondrial fusion. However, a recent structural analysis of mouse mitofusins (Fzo1 homologs) revealed the presence of hydrophobic heptad repeats in these proteins that promote the formation of mitofusin dimers (*10*). Like the heptad repeats present in SNARE proteins, the mitofusin heptad repeats form coiled-coil structures that connect the mitofusins of two adjacent mitochondria (see the figure). SNARE proteins pair in a parallel fashion to bring two vesicle membranes into close apposition (*9*). In contrast, mitofusins form an antiparallel coiled coil, leaving a gap of about 10 nm between the two mitochondria (*10*). Thus, the GTP-independent dimerization of Fzo1 molecules is required for the initial tethering of mitochondria; subsequent fusion events are likely to be promoted by GTP-dependent steps involving Fzo1, Ugo1, and Mgm1. The in vitro fusion assay faithfully reflects this requirement because the formation of Fzo1 homodimers on opposing mitochondria is required for matrix mixing to take place (*5*).



Mitochondrial fusion dissected. Fusion of mitochondria requires the sequential interaction of outer and inner membranes. Fusion of the outer membranes of two adjacent mitochondria requires low GTP levels, whereas the subsequent fusion of the inner membranes requires high GTP levels and the presence of an inner-membrane electrical potential (*5*). (**Box**) Three components of the mitochondrial fusion machinery are known: the outer-membrane GTPase (Fzo1), the intermembrane-space GTPase (Mgm1), and the outer-membrane protein Ugo1 that links both GTPases. Formation of Fzo1 homodimers between adjacent mitochondria promotes their initial tethering. Subsequent fusion of the two mitochondria requires the cooperation of Fzo1, Ugo1, Mgm1, and additional fusion proteins and regulatory factors.

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Mitochondrial fusion requires strict coordination and regulation because four membranes have to fuse, but the specificity of the mitochondrial subcompartments must be maintained. With their in vitro assay, Meeusen *et al*. have identified the intermediate stages of mitochondrial fusion, which has enabled them to gain mechanistic insight into the factors regulating mitochondrial morphology. Further in vitro assays can now be conceived for elucidating other processes that are critical for mitochondrial morphology, including the opposing fission reaction.

This is not the end of the story, however. There are other questions that need to be addressed. For example, which of the proteins found in genetic screens (*4*) and in the mitochondrial proteome (*3*) are directly involved in mitochondrial fusion, fission, or movement, and which are regulatory or supportive factors? Two recent findings provide an interesting link between the protein import and assembly machinery and the maintenance of mitochondrial morphology. First, Mgm1 is present in two isoforms of different length, both of which are required for fusion (*11*). The long isoform is partially imported into the inner membrane and is cleaved to the shorter isoform by a rhomboid protease. The ratio between the two isoforms is controlled by the activity of the presequence translocase-associated motor PAM. When PAM is impaired—for example, in the presence of low adenosine triphosphate levels—cleavage to the short isoform is reduced, leading to fragmentation of mitochondria (*12*). Thus, the energetic state of mitochondria may participate in controlling mitochondrial morphology. Second, Mdm10 (mitochondrial distribution and morphology protein) is required for both maintenance of mitochondrial morphology and transfer of mitochondria to daughter cells (*1*, *6*). Surprisingly, Mdm10 is a subunit of the protein sorting and assembly machinery (SAM) of the outer membrane (*13*). Defects in Mdm10 impair the assembly of the general protein import channel of the outer membrane. Mdm10 and other factors implicated in mitochondrial morphogenesis may thus influence morphology in a SAM-like manner by controlling the assembly of functional protein complexes.

The machinery for maintenance of mitochondrial morphology should not be viewed as a separate entity but instead as machinery that is connected to other important mitochondrial functions such as bioenergetics, DNA inheritance, and protein import, assembly, and turnover (*1*, *2*, *4*, *6*). It is also possible that proteins may play dual roles in morphology and other mitochondrial processes. The challenge will be to dissect the complex process of changing mitochondrial morphology into distinct steps in order to analyze the functional contributions of individual proteins. Like the in vitro assays that have been established for mitochondrial protein import and assembly, the fusion assay of Meeusen *et al.* is likely to pave the way for developing other in vitro assays. Together these assays will be able to unravel the molecular events underpinning the dynamic morphology of these double-membraned organelles.

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CHEMISTRY

Japan Bats a Triple

Robert West

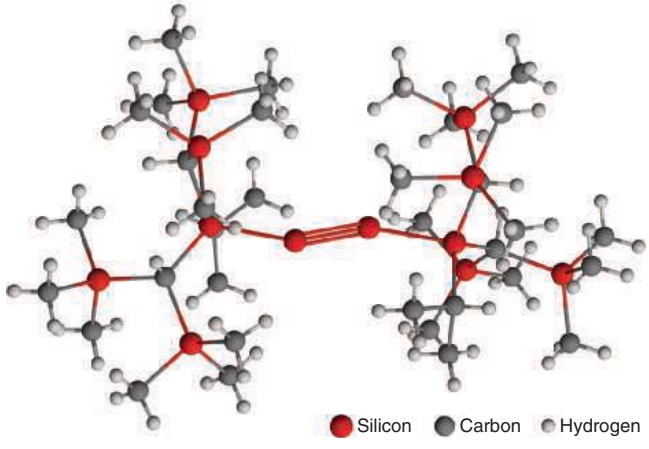
Double and triple covalent bonds are standard in carbon chemistry but are unusual for heavier elements, including carbon's closest neighbor in the periodic table, silicon. On page 1755 of this issue, Sekiguchi *et al*. (*1*) report the achievement of a long-sought goal: the synthesis and full characterization of the first compound with a silicon-silicon triple bond (see the figure) (*1*).

Triple bonds to silicon, the element most closely related to carbon, have been a major target of research for nearly 20 years. Theoretical calculations have suggested that it should be possible to stabilize silicon-silicon and silicon-carbon triple bonds through the use of appropriate chemical groups attached to the atoms forming the bond (*2*). However, until this week's report (*1*), laboratory evidence had eluded chemical researchers around the world. Behind the impressive discovery reported by Sekiguchi *et al.* lies a fascinating sequence of investigations.

By the late 19th century, multiple bonds between carbon atoms and between carbon and other light elements (such as oxygen and nitrogen) were well established as vital features of organic chemistry. Chemists next began to search for multiple bonding among the heavier elements silicon, germanium, phosphorus, arsenic, and antimony. In the early 20th century, syntheses of some doubly bonded compounds involving these elements were claimed. However, upon close examination, all such efforts were shown to lead to singly bonded cyclic oligomers or polymers, not multiply bonded compounds (*3*).

Early theoretical papers by Pitzer (*4*) and Mulliken (*5*) rationalized these results, showing that multiple bonds between heavier elements should be weak. Multiple covalent bonds will form only if *p* orbitals on the two atoms overlap perpendicular to the axis between them, resulting in a π bond. Such bonds are energetically favorable for small atoms, but the π overlap and hence the bond strength decrease rapidly as the atoms become larger.

The combined experimental and theoretical results led to the consensus expressed in the "double bond rule," which



The molecule with the first silicon-silicon triple bond (*1*).

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states that elements outside the first row of the periodic table do not form multiple bonds either with themselves or with other elements. This view was presented in most chemistry textbooks until the 1980s. The rule collapsed, however, on 28 March 1981, when, at the end of the 15th annual North American Organosilicon Symposium in Durham, North Carolina, two speakers announced the discovery of stable chemical compounds containing silicon-carbon (*6*) and silicon-silicon (*7*) double bonds. The resulting paradigm shift shook the world of silicon chemistry and led to the burgeoning chemistry of multiply bonded compounds of the heavier elements, which today is not only continuing but gaining momentum.

Key to the discovery of stable compounds containing Si=C bonds (silenes) and Si=Si bonds (disilenes) was the protection of the double bonds by bulky substituent groups, which provide both kinetic and thermodynamic stability. This strategy has also been used to synthesize other doubly bonded compounds of the heavier elements. Stable doubly bonded compounds are now well established for almost all the elements in periodic groups 13, 14, 15, and 16, and reactions involving these compounds have led to a rich and growing field of chemistry.

With the question of double bonds between heavier elements largely settled, attention turned to triple bonds. Phosphaalkynes, with P≡C triple bonds, are well known (*8*), and one example of an arsenic-carbon triply bonded compound has been reported (*9*). Dicoordinate compounds of germanium, tin, and lead with formal triple bonds have been prepared in a series of elegant studies by Power (*10*). However, the latter compounds show marked lone-pair character and decreasing π-overlap on the bonded atoms, reducing the bond orders from 3 (a triple bond) to about 2 (a double bond) for germanium and tin and 1 (a single bond) for lead.

Sekiguchi *et al.* first announced their discovery at the 38th North American Organosilicon Symposium in May 2004, 23 years after the first double bonds to silicon were reported. Their disilyne is obtained as emerald-green crystals and is stable up to 127°C. The triply bonded silicon atoms are sterically protected by extremely bulky substituent groups (see the figure). The distance between the central silicon atoms is consistent with a true Si≡Si triple bond, as is the bond order. In sharp contrast to the C≡C triple bond in acetylenes, the two Si-Si π bonds in the new molecule are not equivalent.

The synthesis of a stable disilyne is a milestone both for silicon chemistry and for multiple-bond chemistry in general. Further examples of disilynes are likely to be synthesized, and studies of their chemical bonding and chemical reactions should provide a new chapter in silicon chemistry. Meanwhile, the syntheses of compounds with silicon-carbon and silicon-nitrogen triple bonds remain as challenges for the future.

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CHEMISTRY

A Dash of Proline Makes Things Sweet

Erik J. Sorensen and Glenn M. Sammis

Hexose carbohydrates come in many forms, including the common sugar glucose, and are integral to biological processes as diverse as cell signaling, inflammation, the immune response, and tumor cell metastasis (*1*). Each function is specific to a particular form of hexose, but studying how structure relates to function is limited by the difficulty of obtaining pure samples of the hexoses and their derivatives. The de novo construction of complex, differentiated carbohydrates by chemical synthesis is feasible but challenging owing to the structural and stereochemical complexity of these polyhydroxylated compounds. The ideal strategy for synthesizing the hexose carbohydrates would join three simple, α-oxygenated, two-carbon aldehydes by sequential aldol addition reactions with high enantio- and diastereoselectivity. On page 1752 of this issue, Northrup and MacMillan (*2*) describe remarkable, two-step, stereoselective syntheses of several polyol-differentiated hexoses from achiral two-carbon building blocks. A dash of the amino acid L-proline (**1**) and classical organic chemical reactivity are the keys to this powerful new synthesis of these six-carbon sugars.

Enhanced online at www.sciencemag.org/cgi/content/full/305/5691/1725

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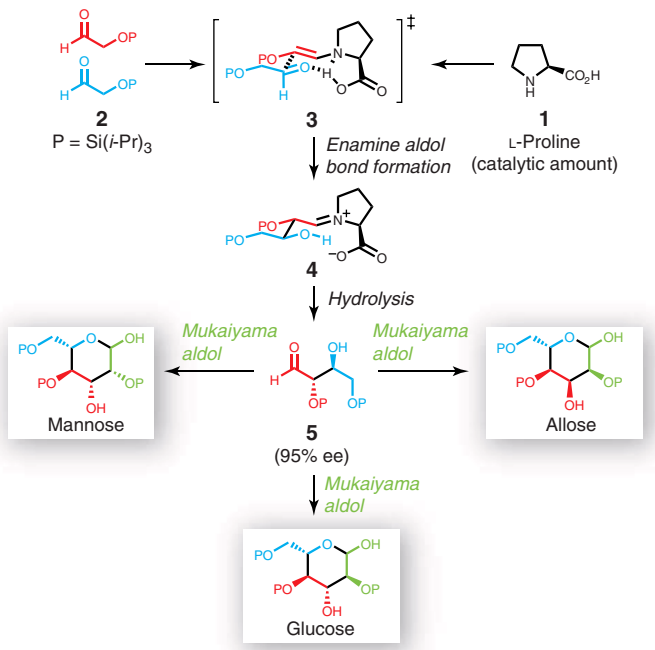
The Northrup-MacMillan route to the hexoses exploits proline's tendency to condense with carbonyl compounds, a reaction that is fundamental in nature and chemical synthesis. The nitrogen atom of proline can attack the electrophilic carbonyl carbon of aldehydes and ketones. Collapse of the tetrahedral carbinolamine intermediate releases a molecule of water and yields an electrophilic iminium ion. Although an iminium ion can be attacked by nucleophiles, it can also lose a proton to a suitable base to give an enamine, which has a nucleophilic carbon and thus is a particularly useful reagent in organic synthesis. In the 1950s, the Stork laboratory demonstrated that enamines, especially pyrrolidine-derived enamines, undergo carbon-carbon bond–forming reactions with a range of alkyl halides and acid chlorides (*3*). L-Proline (**1**) is a chiral pyrrolidine and an extraordinarily useful catalyst for stereoselective aldol, Mannich, and various oxidative reactions (*4*). It condenses with aldehydes such as **2** to produce a new bifunctional reactive species **3** that can activate the carbonyl group of another molecule of **2** for the crucial enamine aldol bond formation. The water molecule released in the formation of the proline-derived enamine hydrolyzes the iminium ion **4**, which generates the desired aldol dimer **5** as the major component of a 4:1 mixture of diastereomers, and returns the proline catalyst to the reaction medium for another turn through the catalytic cycle. The enantioselectivity observed in the proline-directed synthesis of **5** is excellent (*5*).

A remarkable and useful feature of the Northrup-MacMillan route to the hexoses is that the initial proline-catalyzed aldol dimerization of **2** stops at the stage of the four-carbon aldehyde **5**. Although it is not entirely clear why the reaction stops at **5**, it may be that an internal hydrogen bond diminishes the affinity of the carbonyl oxygen of **5** toward the type of Brønsted acid activation shown in **3**. Compound **5** resists further reaction with proline, but it can be activated with oxophilic Lewis acids such as $MgBr_2$ or $TiCl_4$ for a diastereoselective Mukaiyama aldol addition reaction (*6*)



Northrup-MacMillan route to the hexoses. L-Proline–catalyzed enamine aldol bond formation (**2** → **4**) is followed by tandem Mukaiyama aldol bond formation and cyclization to form differentially protected hexoses.

with an enol silane derived from an α-oxygenated aldehyde **2**. This type of aldol reaction generates a new carbon-carbon bond, two new oxygen-bearing stereocenters, and a transient oxocarbenium ion that triggers the final cyclization to the pyran ring of the hexoses. The stereochemical course of this final aldol addition is tunable by simple adjustments in the experimental conditions. Simple two-carbon aldehydes and sequential proline- and Lewis acid–mediated aldol reactions are the keystones for the most expedient syntheses of these hexoses reported so far.

This is one of several important recent advances in the field of catalytic asymmetric synthesis that is based on the chemistry of the amino acid proline (*4*, *7*). This renaissance in the use of proline in asymmetric catalysis is rooted in the early studies of enamine chemistry by Stork and co-workers (*3*) and the pioneering research on proline-catalyzed aldol cyclizations by Hajos, Parrish, Eder, Sauer, and Wiechert (*8*, *9*). Asymmetric enamine aldol additions are also at the heart of the mechanisms of the class I aldolases (*10*) and certain catalytic antibodies (*11*). These findings have inspired recent innovations featuring proline-based asymmetric organocatalysis. The important studies of List *et al*. (*12*) and Northrup *et al*. (*5*) demonstrate that proline, with a molecular weight of only 115, provides access to the world of enamine-centered asymmetric catalysis and is capable of effecting clean and stereoselective aldol addition reactions.

There is great power in the experimentally straightforward process described by Northrup and MacMillan (*2*) because it rapidly produces diverse, differentiated hexoses, even fully ^{13}C-labeled hexoses, for research in glycobiology, medicinal chemistry, and metabolomics. This new synthesis affords an important type of molecular complexity from commonplace molecular building blocks.

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BIOMEDICINE

Eosinophils in Asthma: Remodeling a Tangled Tale

Marsha Wills-Karp and Christopher L. Karp

The ongoing epidemic of allergic asthma in the developed world has lent urgency to the quest to understand the pathogenesis of this chronic, often debilitating, inflammatory disease. There is a broad consensus that allergic asthma is a maladaptive immune response to otherwise harmless inhaled substances in genetically susceptible individuals. This elegantly vague formula hides considerable ignorance and controversy. For example, there is heated debate about whether white blood cells called eosinophils are involved in the pathogenesis of asthma. Although the presence of eosinophils at sites of allergic inflammation was recognized in the 1800s with the first pathological description of fatal status asthmaticus (*1*), the experimental elucidation of the part played by these cells in allergic asthma has been convoluted. Two papers in this issue, by Lee *et al*. (*2*) on page 1773 and Humbles *et al*. (*3*) on page 1776, revisit this controversy using elegant transgenic techniques. Both studies suggest that eosinophils are an integral part of experimental allergic asthma, but apart from this common finding they report divergent results. This divergence suggests complexities that will keep the asthma research field contentious and lively for many years to come.

The cardinal features of allergic asthma include elevated concentrations of serum immunoglobulin E (IgE), pulmonary eosinophilia, airway hyperresponsiveness, excessive airway mucus production, and airway remodeling marked by peribronchiolar collagen deposition and increases in airway smooth muscle mass. As with other allergic diseases, asthma is clearly dependent on $CD4^+$ T lymphocytes that are skewed to a T helper cell 2 (T_H2) phenotype (*4*). Indeed, T_H2, cytokines such as interleukin-4 (IL-4), IL-5, IL-9, and IL-13 drive asthma pathogenesis. The evidence for this is that (i) IL-4 drives the T helper response in favor of T_H2, resulting in enhanced production of IgE; (ii) IL-5, along with GM-CSF (granulocyte-macrophage colony-stimulating factor) and IL-3, is important for the production of eosinophils; (iii) IL-13 is required for airway hyperresponsiveness and mucous metaplasia, the downstream pathophysiological features most closely linked with clinical asthma;

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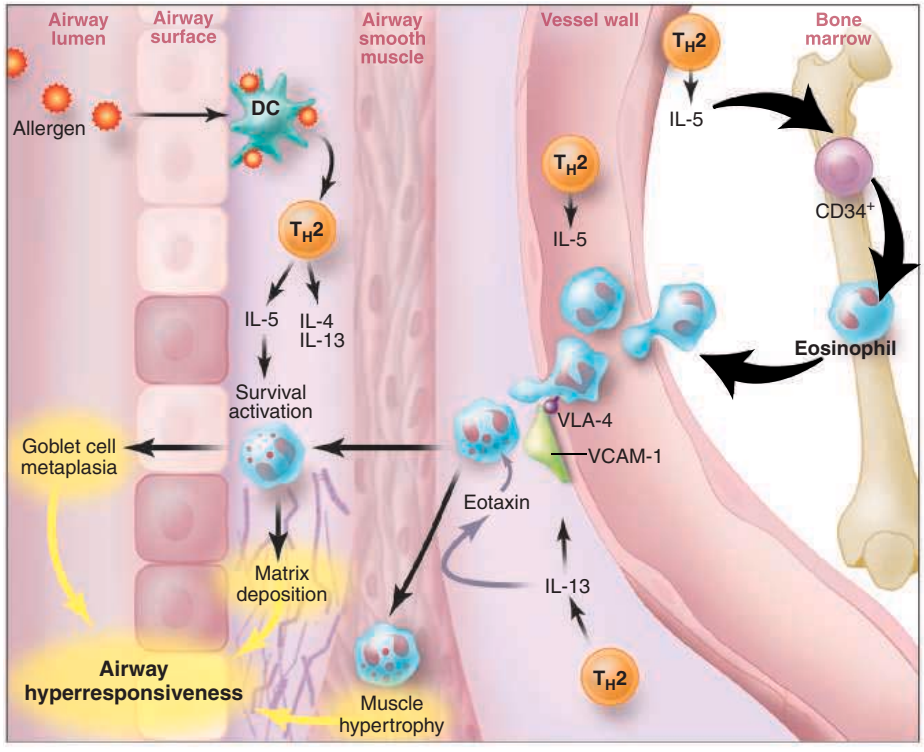
and (iv) all of these cytokines, together with TGF-β (transforming growth factor–β), have been implicated in airway remodeling. Thus, T_H2 lymphocytes and the cytokines they produce are key players in allergic asthma. But do eosinophils, the ubiquitous companions of T_H2 cells, also play a part in asthma pathology, or are they unjustly maligned bystanders?

A role for eosinophils is plausible given their ability to secrete both cytotoxic (eosinophil peroxidase) and bronchoactive (leukotrienes) mediators (*5*). Eosinophils can also present antigens and secrete T_H2 cytokines (*6*), perhaps amplifying or perpetuating T_H2 inflammatory processes at sites of disease. Despite this promising repertoire of immune regulatory and effector properties, pulmonary eosinophilia has often been separable from disease pathophysiology in mouse models of allergic asthma. For example, neutralization of IL-13 effectively blocks allergen-driven airway hyperresponsiveness and mucous metaplasia without substantially altering the number of eosinophils in the lungs (*7*). To date, experimental depletion of eosinophils through antibody-mediated or genetic targeting of IL-5 has yielded conflicting results: both pronounced and negligible effects on asthma pathophysiology (*8*–*10*). Because eosinophils are not completely eliminated by such depletion strategies, these results have been questioned.

In asthma patients, the number of airway eosinophils is associated directly with disease severity. Effective corticosteroid therapy is associated with a reduction in the numbers of such cells. But guilt by association is not enough. Following work in mouse models, therapeutic trials of eosinophil depletion using humanized monoclonal antibodies against IL-5 are under way. In an initial randomized, double-blind, placebo-controlled study of mild asthmatics, a single dose of anti-IL-5 antibody reduced the number of eosinophils in both blood and sputum without appreciably altering airway function or disease symptoms (*11*). The study design leaves open the question of whether a complete and sustained reduction in eosinophils is necessary for therapeutic benefit. Notably, a follow-up study using a multiple-dosing regimen led to the attenuation of several indices of airway remodeling, including matrix protein deposition and airway smooth muscle hypertrophy. However, there were no beneficial effects on airway physiology or symptoms in the treated asthmatics (*12*). The experimental and therapeutic controversy continues.

Bona fide eosinophil-deficient mice have now been generated successfully using two different transgenic strategies (*2*, *3*). Humbles *et al*. generated mice with targeted mutations in the gene encoding GATA-1, a transcription factor essential for the erythroid and megakaryocytic differentiation of immature myeloid cells. Whereas genetic deletion of GATA-1 leads to embryonic lethality, deletion of a high-affinity GATA-1 binding site in the GATA-1 promoter (Δdbl GATA) leads to selective ablation of the eosinophil lineage (*13*). In a different approach, Lee *et al*. targeted eosinophils through transgenic expression of the diphtheria toxin A chain under control of the eosinophil peroxidase promoter (*PHIL*). Both types of transgenic mice lack eosinophils, and that's where the similarities end. The Δdbl GATA mice still show airway hyperreactivity and excess mucus production, the "acute" pathophysiological manifestations of asthma. Such mice, however, also show attenuation of airway remodeling. We note that such findings are congruent with the results of trials using IL-5–specific antibodies in humans. On the other hand, *PHIL* mice are completely protected from developing airway hyperresponsiveness and show partial protection from airway mucous metaplasia. The authors did not report whether *PHIL* mice also exhibit diminished airway remodeling. The authors allude to data indicating that T_H2 cytokine production is suppressed during experimental asthma in *PHIL* mice (which was not reported in the Δdbl GATA mice). Such findings suggest an obvious way in which eosinophils could promote asthma pathophysiology, that is, through production of T_H2 cytokines (for example, IL-13) at the site of disease.

What explains the differences in these



Eosinophils and asthma. Presentation of allergens at the airway surface by antigen-presenting cells (dendritic cells) results in T_H2 cell differentiation and T_H2 cytokine production (IL-4, IL-13, and IL-5) in asthmatic individuals. $CD4^+$ T cells orchestrate the ensuing inflammatory response characterized by an influx of eosinophils into the airway spaces in response to cytokines. Eosinophils are recruited into the airway spaces from the bone marrow through several steps coordinated by T_H2 cytokines. First, their development in the bone marrow from $CD34^+$ progenitor cells occurs under the influence of cytokines (IL-5, IL-13, and GM-CSF) produced by $CD4^+$ T cells. Once cells are committed to the eosinophil lineage, they move from the bone marrow into the vasculature under the guidance of IL-5. Their movement through the blood vessel walls into sites of T_H2-mediated inflammation is controlled by IL-4 and IL-13. These cytokines induce expression of vascular cell adhesion molecule–1 (VCAM-1), which binds to its receptor (VLA-4) on the surface of eosinophils. This leads to the preferential extravasation of eosinophils through vessel walls into sites of inflammation. Once activated by IL-5 or chemokines (eotaxin), eosinophils may contribute to the pathogenesis of allergic asthma by acting on various cells in the airway walls (fibroblasts, smooth muscle cells, and epithelial cells) or by secreting additional T_H2 cytokines. Under certain circumstances, these effects culminate in enhanced sensitivity of the airways to various stimuli, resulting in airway hyperresponsiveness, overproduction of mucus, increased mucus secretion, and deposition of collagen around the basement membrane.

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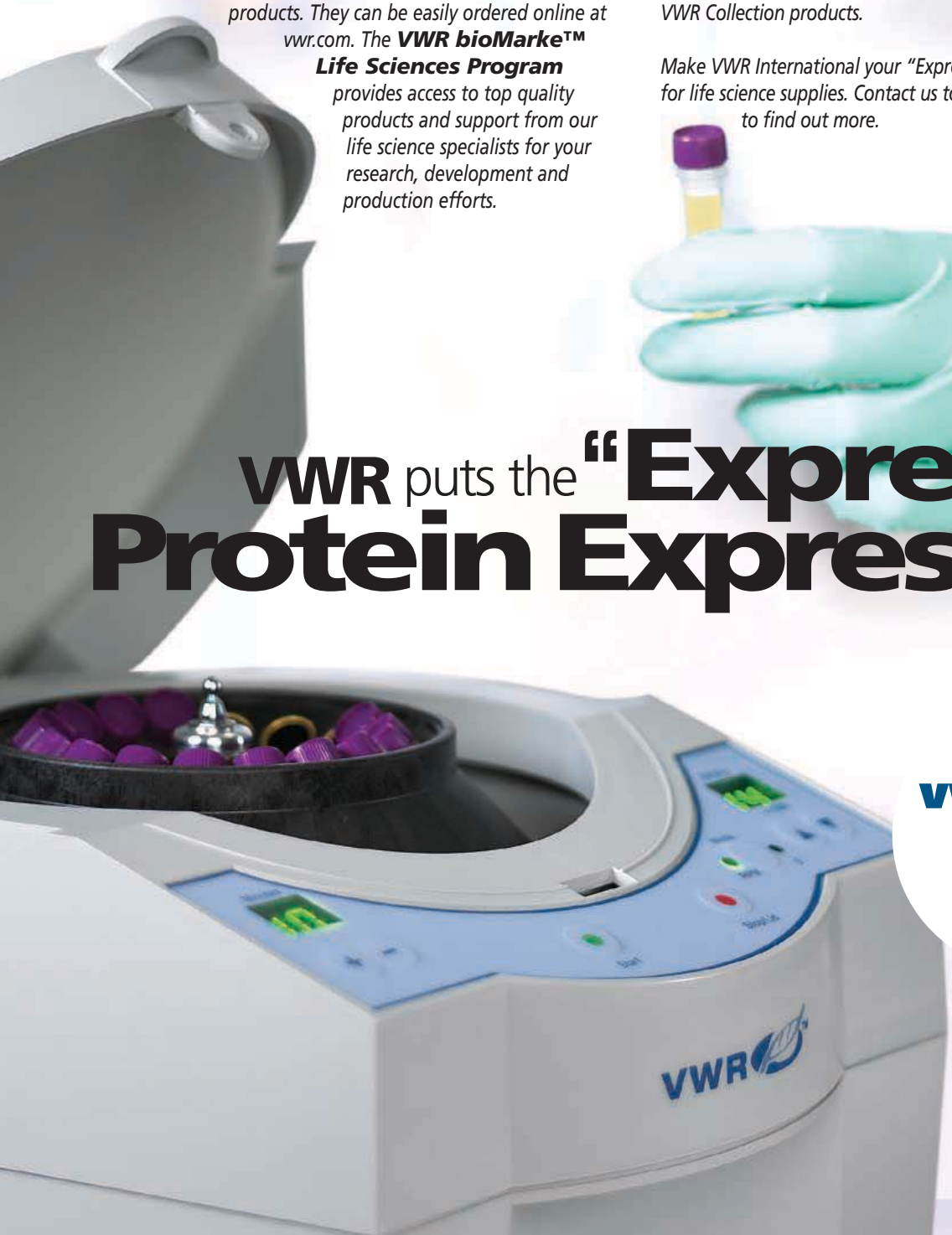

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two studies? Why are eosinophils critical to acute asthma pathophysiology in one strain but not the other? One obvious explanation is background strain variability. Allergic asthma is known to be a multigenic syndrome in both mice and humans. The Δdbl GATA mice were bred on a Balb/c background, whereas the *PHIL* mice were bred on a B6 background. In congruence with the current findings, IL-5–deficient mice on a Balb/c background are protected from airway hyperreactivity, whereas IL-5–deficient mice on a B6 background are not (*9*, *14*). Of course, it remains possible that the method of eosinophil deletion in *PHIL* mice leads to effects outside of the eosinophil lineage, something hinted at by the >50% increase in peripheral blood leukocytes seen in such mice. Might diphtheria toxin–mediated apoptotic deletion of maturing eosinophils (for example, in lymph nodes) (*6*) also have broad immunosuppressive effects (*15*)? In any case, crossing the two transgenes onto the reciprocal mouse backgrounds should provide a definitive answer.

The current studies point to a role for eosinophils in experimental allergic asthma. Taken together, however, the new findings suggest that, although eosinophils may be essential for airway remodeling, they may not contribute to airway hyperreactivity or mucous metaplasia. Although such complexities may dismay partisans of the plucky eosinophil, they provide a satisfying experimental mirror of the multigenic substrates and phenotypic heterogeneity exhibited by patients with allergic asthma. Targeting eosinophils may well provide effective therapy for a subset of asthma patients. More prosaically, the elegant transgenic mouse models presented in these two studies should lead to new insights into the part played by eosinophils in other allergic disorders, parasitic infections, and cancer.

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PLANETARY SCIENCE

Predicting the Sun's Oxygen Isotope Composition

Qing-zhu Yin

Most inner solar system materials have distinct oxygen isotopic compositions (see the figure, inset). A more pronounced variation is observed for some high-temperature components of the most primitive meteorites (see the figure), which represent the most pristine samples of the solar nebula from which our solar system formed. Clearly, the solar system has ^{16}O-rich and ^{16}O-poor reservoirs (*1*), but exactly what this observation tells us about the early solar nebula remains elusive. Part of the difficulty is that the oxygen isotope composition of the Sun—which represents 99.87% by mass of the solar system—is not known with sufficient precision to determine how the planetary materials evolved from the bulk reservoir.

Recently, a new class of models (*2*–*4*) has been proposed to resolve this long-standing problem. In contrast to earlier models that concentrated on the symmetry of minor gas species such as O_3, O_2, and CO_2 (*5*), the new models focus on photochemical isotopic effects on major volatile species such as CO (second in abundance to H_2 in molecular clouds). On the basis of such a model, Yurimoto and Kuramoto postulate on page 1763 of this issue (*4*) that oxygen isotope fractionation occurred in the molecular cloud that collapsed to form the solar nebula. They also explain how these isotopic effects may have been transported into the primitive meteorites (called chondrites) of the inner solar system.

The new model (*4*) tracks a parcel of dust and gas in a molecular cloud with an initially homogeneous oxygen isotopic composition. When the molecular cloud is subjected to ultraviolet (UV) radiation from an external source such as a nearby star, photodissociation of CO occurs. The most abundant isotopomer, $^{12}C^{16}O$, quickly consumes all UV photons that have the appropriate energies for dissociating it, and its dissociation therefore stops at the cloud surface. Dissociation of less abundant isotopomers such as $^{12}C^{17}O$ and $^{12}C^{18}O$ requires UV photons with slightly different energies. Because these photons are not consumed as quickly by the less abundant species, dissociation of the latter continues deeper inside the molecular cloud. This "UV self-shielding" is widely known to occur on the edge of molecular clouds. It generates ^{17,18}O-rich atomic oxygen and ^{16}O-rich CO inside the molecular cloud.

In the dense, cold core of the molecular cloud, the ^{17,18}O-rich atomic oxygen rapidly reacts with hydrogen to form a water-ice mantle on silicate grains. The grains retain the original average oxygen isotope composition of the molecular cloud, whereas the mantles are ^{17,18}O-enriched. When the molecular cloud collapses and the young stellar object ignites, its surroundings are heated through radiation and/or shocks that will chemically and thermally process the ice-mantled grains, producing the observed isotopic heterogeneity along the $\delta^{17}O/\delta^{18}O$ line with slope 1 (see the figure) (*6*).

Clayton (*2*) recently suggested that the UV source for self-shielding may have been the nascent Sun itself. Because the nebula was very dense and opaque, UV irradiation is thought to have occurred only very near to the young Sun (*7*). In this scenario, it is unclear how the chondrite matrix (which, in contrast to some inclusions, was never processed above 400 K) inherited its ^{17,18}O-rich signature or how most of the water in the solar system became ^{17,18}O-rich near the Sun and were transported to the outer solar system.

Lyons and Young (*3*) suggest that CO was dissociated at the disk surface of the solar nebula, further away from the Sun, where the UV source could be either the Sun or other nearby stars. However, it seems that transport of ^{17,18}O-rich water from the disk surface down to the nebular midplane would have taken too long for the oxygen isotopic signature to be locked into the planetary system. Furthermore, their calculated $\delta^{17}O/\delta^{18}O$ slope of 1.05 to 1.10 (*3*) is higher than the observed value of 0.94 to 1.00 (see the figure).

The model of Yurimoto and Kuramoto (*4*) is not subject to such a time constraint, because the ^{17,18}O-rich water is generated in the molecular cloud well before the solar-nebula disk forms. The close association of the icy mantle with the dust grains

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readily explains the ^{17,18}O-rich signatures in the chondrite matrices. However, the authors simply assume that the $\delta^{17}O/\delta^{18}O$ slope is 1 (*4*). Clearly, the exact production ratio of $^{17}O/^{18}O$ in CO self-shielding as a function of UV fluence needs to be determined in the laboratory to better than 5%, because the astronomical observations are too imprecise.

According to the self-shielding models, the oxygen isotopic composition of the bulk solar nebula (hence the Sun) has $\delta^{17,18}O = -50‰$ (see the figure), similar to some minor constituents of chondrites, such as calcium-aluminum–rich inclusions (CAIs), amoeboid olivine aggregates, and some rare chondrules (most chondrules are ^{16}O-depleted). All other inner solar system materials must then have changed by more than 50‰ from the original composition to reach the current values just above 0‰ (see the figure).

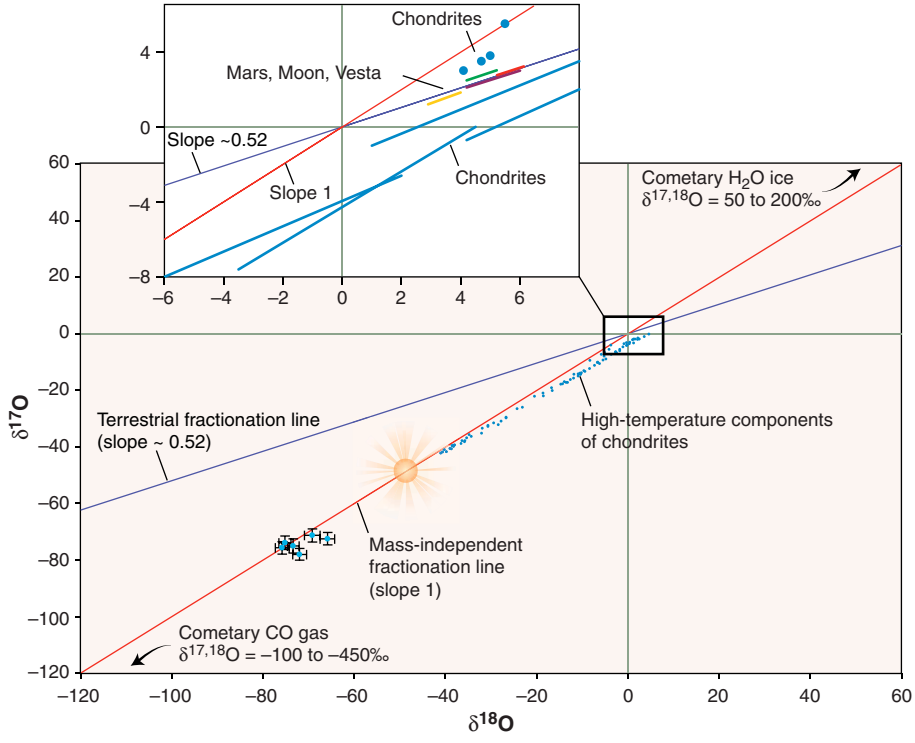
However, some rare chondrules (*8*) have $\delta^{17,18}O$ as low as –70 to –80‰ (see the figure), implying that even CAIs and amoeboid olivine aggregates near –50‰ are not the most pristine representatives of the bulk solar nebula. This raises the question of whether any existing traces of material retain the oxygen isotope composition of the primordial solar nebula.

The self-shielding model depends strongly on the available UV flux: Too much UV will dissociate all CO, whereas too little will not dissociate it at all. In both cases, no isotopic fractionation occurs. Thus, if the model is correct, the observed oxygen isotopic variations put constraints on the total UV fluence in our solar nebula, which may in turn provide information about the astrophysical settings in which our solar system originated. Isotopic effects in elements other than oxygen, such as hydrogen, nitrogen (*9*), and sulfur, may also elucidate the astrophysical site where self-shielding occurs.

Clayton originally proposed that the slope 1 line represents mixing of pure ^{16}O (produced in stellar nucleosynthesis by He burning) with the "normal" oxygen isotope mixture typical for most inner solar system materials (*1*). He now advocates the self-shielding model (*2*), but the nucleosynthetic origin may still be viable. Observed ^{60}Fe concentrations in chondrites (*10*, *11*) indicate that a supernova must have contributed freshly synthesized material to the solar nebula shortly before the birth of the solar system. One presolar grain with almost-pure ^{16}O ($\delta^{17}O = -921 \pm 47‰$ and $\delta^{18}O = -830 \pm 65‰$) has been found in a chondrite (*12*); it probably originated in the supernova. The slope 1 line would then constrain the dilution factor of the interstellar medium by the freshly synthesized supernova material to between 0.01 and 5%.

A verdict on the predicted solar oxygen isotopic composition would have been expected soon had NASA's Genesis spacecraft returned the solar wind samples safely to Earth as planned on 8 September 2004. The mission aimed to measure the oxygen isotope composition of the solar wind to better than 1‰. However, oxygen isotope measurements on metal grains recovered from lunar soil—exposed to the solar wind for 4 billion years, instead of just 884 days—indicate an extreme ^{17,18}O enrichment of up to 80‰ (*13*), opposite to the prediction of the self-shielding models (*2*–*4*). This observation highlights potential challenges to determining the solar oxygen isotopic composition from solar wind samples.

NASA's Stardust spacecraft collected interstellar dust particles in May 2000 and 2002. In January 2004, it encountered comet Wild 2 and collected at least 1000 cometary particles. Once returned to Earth in January 2006, these samples may become the most important asset to study the link between interstellar and nebular chemistry. NASA has also just launched the "Oxygen in the Solar System" initiative aimed at deriving an overarching model to elucidate the origins of isotopic complexity. We may not have the final answer to what is arguably the most fundamental outstanding problem in cosmochemistry, but we will soon learn a great deal.



Three-oxygen isotope diagram. $^{17}O/^{16}O$ and $^{18}O/^{16}O$ ratios of samples, normalized to Standard Mean Ocean Water (*6*). Most planetary materials are clustered near the origin (**inset A**), subparallel to the terrestrial fractionation line defined by the mass-dependent relation $\delta^{17}O = 0.52\ \delta^{18}O$. High-temperature components of chondrites (such as CAIs, amoeboid olivine aggregates, and chondrules) follow the mass-independent fractionation line with slope 1. Some chondrules have extreme values of $\delta^{17,18}O_{SMOW}$ around –80‰ (data points with error bars) (*8*). Data are from (*1*, *8*, *14*). Oxygen isotope compositions of the Sun, cometary H_2O, and cometary CO are according to (*4*).

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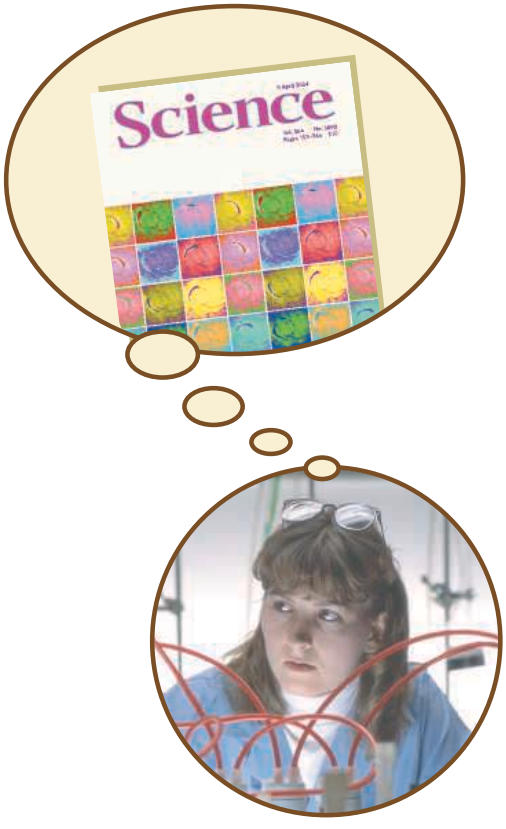

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Living with the Past: Evolution, Development, and Patterns of Disease

Peter D. Gluckman[1]* and Mark A. Hanson[2]

Epidemiological observations have led to the hypothesis that the risk of developing some chronic noncommunicable diseases in adulthood is influenced not only by genetic and adult life-style factors but also by environmental factors acting in early life. Research in evolutionary biology, developmental biology, and animal and human physiology provides support for this idea and suggests that environmental processes influencing the propensity to disease in adulthood operate during the periconceptual, fetal, and infant phases of life. This "developmental origins of health and disease" concept may have important biological, medical, and socioeconomic implications.

It has been proposed that the risk of suffering chronic diseases depends in part on environmental influences acting early in life (*1*). The "developmental origins of health and disease" model arose largely from retrospective epidemiological studies of human populations (*1*–*3*). The relative size and importance of such developmental and nongenetic effects have been disputed (*4*, *5*). We review the clinical and experimental data and the mechanisms involved, and evaluate the wider implications arising from this concept.

Epidemiological and Clinical Studies

Retrospective epidemiological analysis of causal factors in a disease process spanning most of a lifetime is challenging because concurrent risk factors carry greater weight and it is difficult to identify or attribute risk to distant, early-life factors. In addition, direct study of the potential impact of development on later disease outcomes is difficult because of the need for unbiased cohorts with both perinatal data and health outcomes documented well into middle age. Thus, most studies have used surrogate (i.e., indirect or proxy) measures of disease risk, such as systolic blood pressure or fasting insulin/glucose ratios. Although the definition of the health/disease boundary is inevitably arbitrary, where clinical cardiovascular or metabolic disease is the measured outcome, the effect of early environmental influences is clear (Fig. 1).

There are now many epidemiological studies (*1*–*3*) relating impaired fetal growth (deduced from birth weight or body proportions) to an increased incidence of cardiovascular disease or type 2 diabetes mellitus (T2D) or their precursors: dyslipidemia, impaired glucose tolerance, or vascular endothelial dysfunction. Disease risk is higher in those born smaller who become relatively obese as adolescents or adults (*1*). Interpretation of these studies has led to debate about the magnitude of the effect (*4*), although the only published estimate based upon a long-term Finnish cohort (*3*) suggests it to be substantial. Prospective clinical studies on children born small also provide support for the concept (*6*, *7*).

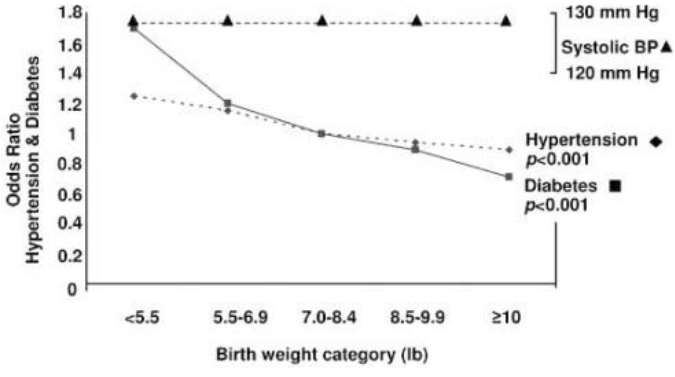

Fig. 1. Data from 22,846 men older than 40 years of age showing strong relationships between birth size and the relative risk of developing clinically significant hypertension or diabetes mellitus but no relationship with systolic blood pressure. These data demonstrate the importance of studying outcomes rather than surrogate measures of disease. Data are derived from G. C. Curhan *et al*. (*2*).

In evaluating the relative role of genetic and environmental factors, it is useful to note that birth size has only a small genetic component and primarily reflects the quality of the intrauterine environment. The observed relationship between disease risk and birth size does not imply a causal role of being born small but reflects the sensitivity of fetal growth to adverse intrauterine influences. It is considered that it is the effect of environmental influences acting during early development that is the causal trigger. Indeed, studies reviewed below indicate that adverse developmental influences can affect disease risk without birth size being affected. The term "maternal constraint" encapsulates those environmental factors that influence birth size even in healthy pregnancies, such as maternal size, age, parity, and multiple pregnancy, and various mechanisms limiting nutrient supply to the fetus (*8*). Firstborn offspring show a higher incidence of low birth weight and increased obesity in childhood and adolescence than their subsequent siblings (*9*). Although nutrition has received the most focus (*10*–*12*), other early-environmental factors such as infection, season of birth (*13*, *14*), and smoking (*15*) may have long-term effects.

There is now evidence for such developmental influences in an increasingly wide range of chronic diseases—osteoporosis (*16*), polycystic ovarian syndrome (*17*), mood disorders (*18*), and psychoses (*19*). Much more research is needed in these areas to establish the extent of the phenomenon unequivocally. Perhaps finding markers of early gene-environment interactions will allow definitive clinical data to be obtained.

Studies of famine indicate that the longer-term effects on offspring may depend on the duration and timing of undernutrition and can be independent of birth size (*20*, *21*). Further, there is increasing evidence that fetal development can be affected by nutritional variation within the normal range of western diets (*22*), and unbalanced dieting by mothers in early pregnancy is common. In addition to the embryonic and fetal periods, the postnatal environment and the infant phase may also play a role. For example, both cognitive function (*23*) and insulin secretion (*24*) in childhood are influenced by the type of feeding in

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the premature neonate, who is subjected to higher fat intakes than are experienced in utero. Rapid weight gain and growth in infancy or childhood may be a further compounding factor, even when birth size is relatively normal (*25*–*27*).

Experimental Studies

Experimental evidence that the prenatal or perinatal environment can influence adult postnatal physiology is available in several mammalian species (*12*, *28*). These studies demonstrate that manipulation of the periconceptual (*29*), embryonic, fetal, or neonatal (*30*) environment can lead to altered postnatal cardiovascular and/or metabolic function. Although the environmental triggering cues are not yet fully understood, most manipulations have been dietary and include maternal pan-undernutrition (*10*, *31*), low-protein diet (*11*), or high-fat (*30*, *32*) diet. Furthermore, in the normal nonmanipulated pig (*33*) and guinea pig (*31*), inverse relationships between birth size and later insulin sensitivity, and blood pressure are reported. Maternal glucocorticoid administration produces effects similar to those produced by undernutrition (*34*, *35*). Undernutrition may suppress placental 11-β-hydroxysteroid dehydrogenase type 2, which inactivates cortisol and exposes the fetus to excess maternal steroid; however, induction of long-term effects can occur well before the placenta is formed and independently of changes in glucocorticoid levels.

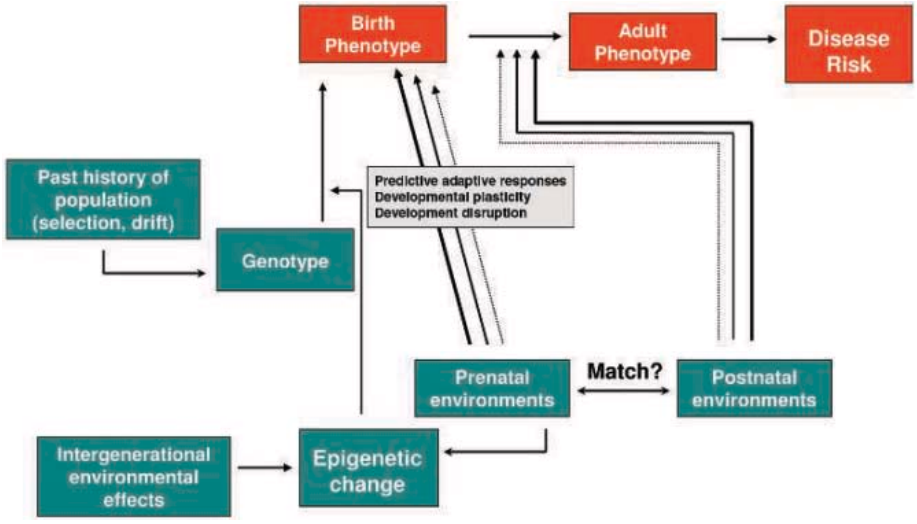

Fig. 2. A general model of how intergenerational, genetic and environmental, and prenatal and postnatal factors interact to create a pathway to altered disease risk in adulthood. If the prenatal and postnatal environments match, the physiological settings achieved through the processes of developmental plasticity will leave the organism well prepared for the postnatal environment. Conversely, a mismatch between the prenatal and postnatal environment may be pathogenic.

Environmental Effects via Developmental Plasticity

"Developmental plasticity" provides organisms with the ability to change structure and function in response to environmental cues; these responses usually operate during critical time windows and then become irreversible. Such plasticity permits a range of phenotypes to develop from a single genotype in response to environmental cues. In *Daphnia*, helmet formation (a defensive, morphological change) is dependent on the early environment and risk of predation (*36*). In the locust, *Locusta migratoria*, the wing shape and metabolic pathways are determined in the larval stage by pheromone signals indicating population density (*37*). In the axolotl, early environmental conditions determine whether the mature form will be purely aquatic or amphibious (*38*). Developmental plasticity sets the template on which continued postnatal homeostatic and homeorhetic [maintaining a time-dependent process, e.g., growth trajectory (*39*)] adaptation can occur.

There are several mechanisms by which environmental cues can influence the developmental program (Fig. 2). First, they can exert effects prior to implantation and affect gene expression, particularly by inducing epigenetic changes in the DNA. In the agouti mouse mutant, maternal dietary folate supplementation at conception alters the expression of the imprinted *agouti* gene by altering the capacity for methylation (*40*). In pregnant rats, giving an additional source of dietary methyl groups prevents vascular defects in the offspring, even if the diet is protein deficient overall. Prolonged in vitro culture of the ovine embryo affects later expression of the imprinted insulin-like growth factor–2 (IGF-2) system (*41*). Nonimprinted genes can also undergo epigenetic change in response to the environment—the choice of exon usage in the glucocorticoid receptor gene is altered both by prenatal glucocorticoids and neonatal behavioral manipulation owing to changes in histone acetylation and DNA methylation in a transcriptional factor binding site (*42*). These changes persist throughout life as manifested in altered hypothalamic-pituitary-adrenal (HPA) axis activity. Intriguingly, the effects could be reversed by a histone deacetylase inhibitor, suggesting potential reversibility. This finding may have broader implications.

Second, tissue differentiation may be altered. Prolonged in vitro culture of the rodent or ruminant embryo affects the allocation of blastocyst stem cells to inner cell mass or trophectoderm lineages (*29*). This influences the relative growth trajectories of the placenta and fetus, thus affecting fetal development in late gestation. Organ-specific effects are also reported. Fetal pancreatic islet cell differentiation is affected by maternal nutritional manipulation, leading to altered developmental apoptosis and expression of transcriptional regulators of the *Pdx* and *Pax* gene families (*43*). Differential expansion of periportal and perivenous hepatocyte cell clones is reported after maternal nutritional or hormonal manipulation and may lead to altered hepatic glucose and lipid metabolism (*44*). Maternal dietary (*45*) or glucocorticoid manipulation (*46*) in the rat reduces the number of renal glomeruli in the offspring. This "trade-off," although it conserves resources in the short term, may induce later glomerular hyperfiltration to maintain fluid and electrolyte homeostasis, and declining renal function with age leads to progressive hypertension. Indeed, this concept of trade-offs between prenatal growth/development and postnatal growth/function may explain the compounding role of rapid postnatal growth in generating additional disease risk (*26*, *27*).

Lastly, changes may be induced in homeostatic control mechanisms. Defects in both insulin secretion, as well as in insulin postreceptor action (*47*) and glucose transporter function in muscle, may predispose the individual to T2D. Vascular endothelial function is altered following maternal dietary manipulation (*48*), and this tissue is involved in controlling blood flow, clotting, inflammation, growth, and metabolism. Intrauterine environmental induction of changes in central nervous cardiovascular control (*49*) is also reported.

These various prenatal changes may alter the offspring's response to the postnatal environment. In rat pups subjected to prenatal undernutrition, exposure to a postnatal high-fat diet has much greater effects on the development of obesity and hyperleptinemia than in pups born to mothers with a normal dietary intake, but then fed a high-fat diet postnatally (*10*). Likewise, pigs exposed in utero to a high-fat diet have a different tolerance to high-fat diets postnatally (*50*).

Types of Response to the Early Environment

Some responses of the embryo or fetus to its environment may, however, be developmentally disruptive with no adaptive value—examples are responses to environmental teratogens. However, the fetus has many homeostatic and homeorhetic mechanisms that confer immediate survival advantage—e.g., alterations in regional blood flows and organ growth when nutrient or oxygen supply is reduced—even if there may be subsequent postnatal costs. A further class of response, termed a predictive adaptive response (PAR) (*28*, *51*), has been identified that appears primarily to have future adaptive value. For example, the coat of meadow vole offspring is thicker if offspring are born at a time of decreasing day length than if they are born in spring, even though the perinatal thermal environments are similar (*52*). It is proposed that PARs allow the developing organism to use developmentally plastic processes to set its postnatal physiological phenotype to one that it predicts will give it an optimal chance of survival to reproduce when an adult.

The responses to an environmental exposure in one generation may extend over several generations and are well recognized in comparative biology as "maternal effects." Their possible role in the developmental origins of disease has been recently reviewed (*53*). For example, birth size is reduced in the offspring of women who themselves were fetuses during transient famine (*54*), and effects on blood pressure, endothelial function, and insulin sensitivity are passed to F_2 offspring of undernourished pregnant rats (*55*). This might reflect either transgenerational passage of environmentally induced epigenetic change, as suggested by studies in the agouti mouse (*40*), or an effect on the reproductive tract of the F_1 generation, as suggested from clinical studies of girls with intrauterine growth retardation (*56*).

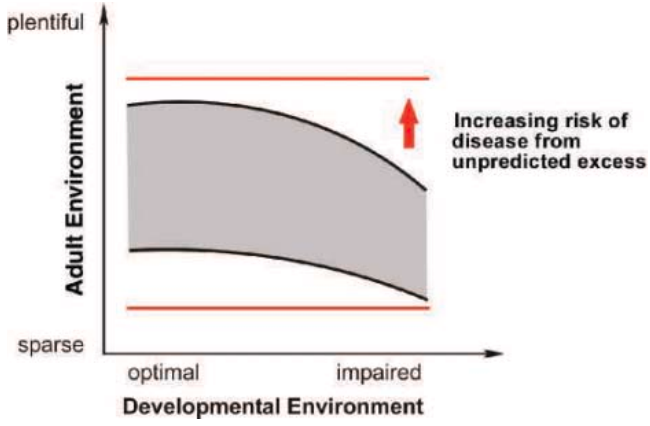

Fig. 3. The red lines show the upper and lower limits of the environmental range (for example, nutrition) to which the mature organism could be exposed. The PARs model proposes that the developing organism growing optimally (left side) adjusts its physiology to be appropriate for its predicted mature environmental range (shaded area). If the early environment is impaired (toward right side), then the range of environments for which the organism is adapted by PARs will not match the mature environment. This mismatch means that the organism is likely to have a physiology inappropriate for the environment in which it is now living (space between the shade area and upper red line). In modern humans, such a mismatch leads to a risk of disease. This scenario is common in the developing world where fetal growth is often constrained by small maternal size, maternal disease, and poor nutrition and where postnatal food availability is increasing. Because the upper limit of the nutritional environment is rising globally, the risk of disease due to mismatch increases even for individuals who had normal early development.

An Evolutionary Perspective

There have been several models proposed to explain the changing demography of "life-style" diseases such as T2D. The "thrifty genotype" concept (*57*) proposed that populations have been selected for alleles favoring insulin resistance. Such "thrifty genes" confer advantage in a poor food/high energy expenditure environment by reducing glucose uptake and limiting body growth. When individuals of this genotype encounter an environment of plentiful food/low energy expenditure, they are at risk of developing T2D and the metabolic syndrome (*58*). So although selection for these genes enabled our ancestors to survive as hunter-gatherers, they put modern humans at greater risk of disease, especially as our longevity increases. Because insulin is a fetal growth factor, selection for such genes might also induce lower birth weight (*5*). For example, mutation in the glucokinase gene produces reduced fetal growth and later insulin resistance independently (*59*).

But purely genetic models cannot explain the reported effects of human famine during gestation (*21*) or the experimental animal studies. The alternate "thrifty phenotype" model (*60*) posits that the fetus becomes growth retarded in response to adverse environmental conditions in utero, and the associated adaptations induce a phenotype better suited to a deprived postnatal food/energy environment. However, this model does not easily account for the graded effects on disease risk seen across the normal birth weight range (*1*, *2*), or the way in which the disparity between the prenatal and postnatal environment determines the level of risk.

Recent work stresses that both genetic and environmental factors must be involved. Studies of a polymorphism in the PPARγ2 gene (which codes for a transcription factor affecting gene expression involved in the control of insulin sensitivity) associated with increased risk of T2D show that the polymorphism is only associated with a higher risk of T2D if birth weight is reduced (*61*). Clearly, the relationship between birth size and disease risk cannot be explained by independent effects on insulin sensitivity and fetal growth. Another example is polymorphisms of the vitamin D receptor, the effect of which is that the risk of osteoporosis is influenced by birth size (*16*).

These various models lead to a more general synthesis (*28*, *51*) (Fig. 3). Developmental responses to environmental stimuli may be either disruptive or adaptive. The former have no evolutionary significance. For the latter, the advantage need not be immediate, but may arise from a PAR made in expectation of the future environment. Such PARs are made during the phase of developmental plasticity to optimize the phenotype for the probable environment of the mature organism, and epigenetic change is likely to be the mechanistic basis (*51*). Where there is a match between the predicted and actual mature environment, these PARs are appropriate and assist survival. Conversely, inappropriate predictions increase the risk of disease. Modeling suggests that such lagged responses aid the survival of a species (*62*).

Longevity was short in ancestral hominids, and thus little negative selection pressure has operated to limit the adverse consequences of a strategy now manifest in modern humans as disease in middle age. However, recent studies charting demographic trends in longevity (*63*) show that such effects are of increasing importance. It appears that evolution has preserved genes encoding mechanisms that allow the organism to mount PARs. A key element of this model is that it focuses on the relative environmental state between the plastic and mature phases. Therefore, because of intrauterine constraint, even when fetal growth falls within the normal range, being born into an enriched postnatal environment can create a mismatch. This model, thus, encompasses both the evidence focusing on embryonic/fetal cues (*1*, *21*, *29*) and infant/childhood cues (*24*, *26*). The importance of the relative nature of the environments in the plastic versus mature phase is demonstrated by the observation that in mice, merely giving adequate postnatal nutrition reduces longevity in prenatally deprived offspring (*64*).

Relevance to Patterns of Disease

The intrauterine environment cannot change dramatically between generations (*51*). But in many societies, high-calorie food is now plentiful and the energy expenditure has become reduced. Thus, the potential for dispar-

ity between pre- and postnatal environments is increasing. In some developing societies, the postnatal food-energy environment has dramatically changed even within a generation, but fetal growth is still markedly constrained; this may explain the rapid increase in the incidence of T2D seen in such populations (*25*).

The experimental and prospective clinical studies add weight to the epidemiological data and suggest that early development does have significant echoes in disease risk throughout life. There is a growing awareness of the potential for epigenetic change to play a role in disease generation. A key issue is the relative importance of early-life events in informing interventional strategies during human development versus those instituted in adult life. If appetite, food choice, and exercise propensity are partially induced during early development as in experimental animals (*30*, *65*), then postnatal life-style interventions may be less effective than hoped. It seems that increasing awareness of the need to promote the health and nutrition of females of reproductive age is one important element for the prevention of chronic disease in future generations across the globe.

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66. M.A.H. is supported by the British Heart Foundation. We thank C. Pinal for her assistance on the manuscript.

Inflammatory Exposure and Historical Changes in Human Life-Spans

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Most explanations of the increase in life expectancy at older ages over history emphasize the importance of medical and public health factors of a particular historical period. We propose that the reduction in lifetime exposure to infectious diseases and other sources of inflammation—a cohort mechanism—has also made an important contribution to the historical decline in old-age mortality. Analysis of birth cohorts across the life-span since 1751 in Sweden reveals strong associations between early-age mortality and subsequent mortality in the same cohorts. We propose that a "cohort morbidity phenotype" represents inflammatory processes that persist from early age into adult life.

A long-term decline in mortality, beginning before 1800 in some countries in Northern Europe, has resulted in a 50% increase in adult life expectancy (*1*, *2*). Childhood mortality has decreased by 90%, and this has been attributed mainly to a decreased incidence of infectious disease (*2*–*4*). After 1850, older-age mortality declined, with greater improvement in recent decades (*1*, *5*). Most explanations of the long-term decline in mortality have focused on improvements in sanitation, nutrition, income, and medicine. We develop the specific hypothesis that decreased inflammation during early life has led directly to a decrease in morbidity and mortality resulting from chronic conditions in old age.

Our argument is supported by recent research linking an individual's exposure to past infection to levels of chronic inflammation and to increased risk of heart attack, stroke, and cancer. For example, the risk of heart attack and stroke is correlated with serum levels of inflammatory (acute phase) proteins such as C-reactive protein (CRP) (*6*–*8*). Within individuals, CRP levels are

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also correlated to the number of seropositivities to common pathogens, indicating a history of infections (*7*, *8*). Furthermore, drugs with anti-inflammatory activities [nonsteroidal anti-inflammatory drugs (NSAIDs), statins] reduce the risk of vascular events and possibly Alzheimer's disease (*9*, *10*), implying links between the levels of inflammation and major chronic conditions important in old age.

Links Between Health in Early Life and in Later Life

Much evidence links early-life infections with later morbidity, including cardiovascular and respiratory disease, cancer, and diabetes. Some infections directly cause organ damage; the classic example is rheumatic heart disease, which increases adult morbidity from heart valve damage incurred from childhood streptococcal infections (*11*). The presence of early-life infections has also been associated with late-life chronic disease and mortality, as shown in many examples from early to recent times. In rural 18th-century Sweden, greater exposure to infections during infancy was associated with higher old-age mortality (*12*). Among U.S. Civil War veterans, infectious disease in early adulthood was associated with heart and respiratory problems after age 50 (*13*). Moreover, respiratory infections in early life were associated with later lung impairments (*12*, *14*). Adult cardiovascular disease has also been associated with cohort levels of infant diarrhea and enteritis (*15*). Among Americans currently in their 50s, those who experienced major childhood illness are 15% more likely to report having cardiovascular conditions and twice as likely to have cancer or chronic lung conditions (*16*). Finally, declining infection during the 20th century has been estimated to explain 11 to 24% of the reduction in late-life morbidity and mortality (*13*).

These associations between early and later morbidity and mortality imply cohort-specific effects on adult mortality trends, especially in old age when vascular conditions and cancer dominate mortality. Cohort effects could arise from the common epidemiological environments that members of a particular birth cohort experience in their younger lives. Thus, changes in the epidemiological environment that occur within a given historical period would affect surviving members of cohorts for the rest of their lives. Enduring effects of early environment, even if conditions improved at later periods, could be designated as a "cohort morbidity phenotype."

As early as 1934, Kermack *et al*. (*17*) noticed that as mortality at younger ages improved in successive cohorts in England and Sweden, the adult survivors in those cohorts also had lower mortality. From this they hypothesized that maternal health and child environment were major determinants of health at later ages, or that the mortality characteristics of a cohort persisted throughout its life-span. In 1956, Jones (*11*) developed further links between cohort mortality at younger and older ages in Swedish 18th- and 19th-century populations by examining mortality rates over the life-span. Although infant mortality was not then available for cohorts born before 1895, Jones concluded that birth cohorts had parallel mortality curves across the adult ages and that mortality curves across the life-span were displaced to lower values in more recent cohorts. He hypothesized that "the physiological age of each new generation is remaining more youthful at the same chronological age" (*11*). This is an important observation, because most biologists and demographers examining mortality change over time at older ages have concentrated on adult mortality.

With the longer data series now available for Sweden, we have updated Jones' observations, graphing age-specific mortality rates for five birth cohorts from 1751 to 1940 (Fig. 1A). The age-specific mortality across the life-span shows progressive downshifts in age-specific mortality for later-born cohorts. Mortality at any given age across the life-span is lower in successive cohorts. Thus, cohorts with lower young-age mortality also have lower mortality at any given age in later life, consistent with Jones' hypothesis.

The cohort analysis also reveals that the major declines in mortality have had little effect on the basic rate of mortality acceleration during aging, as shown for cohorts by parallel linear slopes of mortality on semi-logarithmic plots (Fig. 1A). Data from most human populations show regular relationships of mortality at one adult age to mortality at the preceding age and subsequent ages, in which mortality accelerates more or less smoothly during adult life up to the oldest ages, with a characteristic "Gompertz slope" (*18*). The present cohort data show that major improvements in human environments, which lowered overall mortality by as much as 90%, have not altered the characteristic mortality acceleration during aging. Even transient adversity may shift the line upward, without change in slope (*18*). These progressive reductions in mortality are more resolved when plotted as cohorts rather than as conventional plots of period age-specific mortality over time (Fig. 1B). The similarity of mortality slopes at the older ages observed in the cohort plots is not found in period age-specific mortality curves, which tend to converge at older ages.

Using the Swedish data for individual ages during childhood and old age for persons born in each year from 1751 to 1927, we can further develop two points presaged by Kermack *et al*. (*17*) and Jones (*11*): (i) that the historical mortality decline among the old and young begins in the same cohort, and (ii) that infant mortality has a stronger relationship to later-life mortality than does mortality in subsequent childhood years. Examination of annual trends in childhood and old-age mortality for cohorts born from 1751 to 1927 indicates that declines in mortality after age 70 tend to lag about 70 years behind those for infants (*19*). When we relate childhood mortality to later-age mortality for Swedish birth cohorts born in the 177-year period from 1751 to 1927, we find strong relationships between rates of childhood mortality and mortality for cohort survivors in old age. Most variance in the 177-year series of old-age mortality for cohorts was explained by mortality before age 10. Moreover, the annualized effect of each childhood year on old-age mortality is three times as great for infant mortality than for mortality in subsequent childhood years. This confirms the greater effect of infant versus childhood mortality on old-age mortality observed in a small, rural Swedish region (*12*).

Adult Disease, Infection, and Inflammation

We hypothesize that chronic inflammatory mechanisms drive much of the influence of early-life infections on later morbidity and mortality. As noted above, in contemporary populations, serological indicators of infection and inflammatory indicators are related to vascular disease and many other morbidities of aging. Markers of inflammation include elevations of blood CRP as well as interleukin-6, tumor necrosis factor–α, and fibrinogen (components of the acute phase inflammatory response). Such inflammatory responses can be induced by invading pathogens, as well as by trauma or internal tissue injury. Infections may be local agents in vascular disease, whereas asymptomatic arterial lesions (which are ubiquitous at early ages) appear to progress through inflammatory processes (*20*). Atheromas contain macrophages and other cells that secrete inflammatory proteins, which, if chronically elevated, may be in themselves pathogenic rather than simply indicators of risk. CRP, for example, can activate the complement system and increase low-density lipoprotein uptake by macrophages (*6*). Thus, adaptive responses to short-term infections or injury can become maladaptive in the long term—a double-edged sword that evolutionary biologists refer to as antagonistic pleiotropy.

In the past, substantial proportions of populations in countries that now have low mortality suffered conditions that chronically elevate inflammatory markers. Among populations that still have relatively high mortality, these conditions remain highly prevalent. For

instance, many people living in relatively high-mortality conditions contract chronic tuberculosis, diarrheas, and parasite-borne diseases such as malaria that persistently elevate blood CRP, as observed in tuberculosis (*21*) and infections of *Escherichia coli* and *Helicobacter pylori* (*22*). These associations of CRP with infections suggest that the historical decline in infections would have lowered exposure to CRP and other inflammatory proteins. *H. pylori* is an instructive example. This common bacterium is the major cause of peptic ulcers but is also associated with coronary disease (*23*). *H. pylori* infections are usually acquired in childhood and persist throughout life. In the past, many if not most individuals in what are now low-mortality countries carried *H. pylori*, but because of improved public health and hygiene, infections have declined. For example, *H. pylori* infections decreased by a factor of 6 in birth cohorts of the Bristol *Helicobacter* Project (*24*). Periodontal disease, another source of chronic inflammation, was highly prevalent but has now declined with the introduction of dental hygiene in countries with currently low mortality. Periodontal disease has been associated with chronically high levels of CRP and possibly heart disease (*24*). These and other examples suggest that public health and medical interventions have led to a lower level of diverse infections, which in turn has resulted in reduced inflammation throughout life in modern populations. Thus, the factors that reduce mortality within specific historical periods have also resulted in reductions in lifetime levels of inflammation and old-age mortality within cohorts.

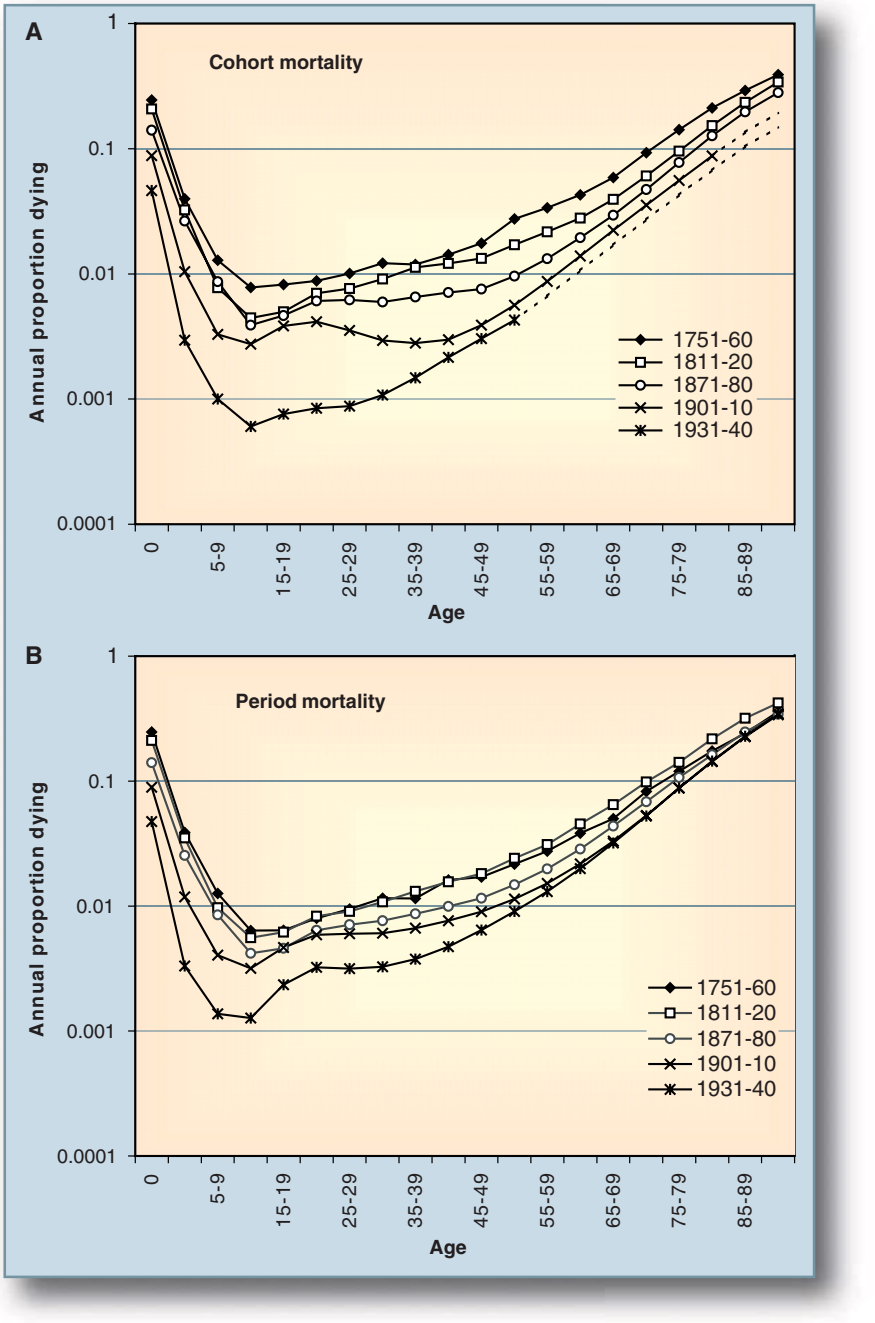

Fig. 1. Age-specific mortality over the life-span, Sweden, 1751 to 1940 (semi-logarithmic plots). Data source: Berkeley Mortality Database. (**A**) Cohort mortality for persons born in the specified years. (**B**) Period mortality occurring in the specified years. Mortality is estimated for the later years of the last birth cohort, assuming that mortality at adjacent age groups has the same relationship as that in the prior cohort, and is represented by a dotted line.

The Role of Nutrition?

Malnutrition at early ages, including in utero, is also hypothesized to influence later-life morbidity and mortality (*2*, *25*). The "fetal origins" hypothesis of Barker *et al*. (*26*) associates low birth weight with maternal malnutrition, leading to higher morbidity at later ages from cardiovascular disease, hypertension, and type 2 diabetes. Attenuated fetal growth is thought to impair organ and vascular development and to alter metabolic set points. Not all evidence supports an exclusive role of malnutrition in the fetal-origins hypothesis. For example, maternal infections from diseases such as tuberculosis, which was common until well into the 20th century, also impair fetal growth (*2*, *4*, *12*). Moreover, the famine in rural Finland from 1866 to 1868 tripled death rates at 0 to 9 years but did not alter the survivors' life-spans (*27*).

We would argue that the inflammatory-infection and nutrition hypotheses are not competing but complementary in linking two mechanisms of morbidity in early and later life: Even well-fed babies are vulnerable to rampant infections, and infections alone can cause malnutrition and later deficiencies. Childhood diarrheas, for example, impair cardiac muscle synthesis (*28*), which could underlie associations of infant diarrhea with later cardiovascular disease (*16*). Slowed infant growth in the Barker hypothesis (*26*) could thus be consequent to infections that cause inflammatory responses as well as impair nutrient absorption. Using historical Swedish cohorts to test both hypotheses, Bengtsson and Lindstrom (*12*) concluded that the level of infection among infants had a stronger influence than food availability on later-life mortality and life expectancy, and they implicated respiratory mechanisms. In addition, we suggest that the systemic inflammatory processes recognized in modern populations are important as risk factors in vascular disease.

Early-life infection may also explain effects of the season of birth on longevity.

Among birth cohorts of the 19th and early 20th centuries from Northern Europe, those born in the spring eventually lived 3 to 6 months longer than autumn births, with corresponding differences in some later-life diseases (*26*). Although seasonal variations in nutrition were emphasized, we would also suggest a role of inflammation.

Conclusions

Long-term declines in mortality for cohorts born in Sweden from the middle of the 18th century into the 20th reveal important links between mortality in old age and in early life. Cohort levels of mortality during childhood are related to cohort mortality in old age, which implies an imprint of early exposures on the cohort morbidity phenotype. Thus, improved public health conditions and medical interventions have both immediate and long-term benefits: a reduction in current mortality combined with the enduring effects of an improved early environment. Our argument implies that mortality in later-born cohorts should be lower over the entire lifespan. Although infection and inflammation are particularly elevated in childhood when infections are very prevalent, chronic infections and lifelong inflammation should characterize all ages in populations with high infectious mortality.

Improved childhood health and survival along with reduced chronic infections and inflammation are attributed to improved public health, medical advances, and an improved standard of living. The links between young- and old-age mortality help to explain the widespread recent declines in old-age mortality and to anticipate further declines in populations where declines of early-age mortality have more recently appeared. The rapid decline in old-age mortality in developed countries that began in the latter part of the 20th century took researchers by surprise. Because few medical breakthroughs occurred before the onset of the decreased mortality from heart disease and stroke in the 1960s, this decline is generally attributed to lifestyle change and medical advances (*29*). According to our hypothesis, these improvements in part originated as cohort effects from reduced early morbidity.

The stability of cohort mortality slopes (Gompertz slope), despite the remarkable variability of overall mortality (Fig. 1A) (*18*), implies that future increases in life expectancy from reduced inflammatory causes may be relatively small, particularly in populations that have had low levels of childhood infection for many decades and now may be approaching a lower limit. These findings from many fields suggest that inflammatory processes that influence the outcomes of aging can be kindled or quenched by exposure to extrinsic infections, inflammatory stimuli, and nutrition. A new theory of human health in life history could emerge from a fuller accounting of inflammatory exposures from gestation to old age.

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30. We thank A. Hagedorn and R. Vejella for data analysis and graphics, and B. Henderson, V. Longo, B. Teter, and A. Watts for helpful comments. Supported by National Institute on Aging (NIA) grants AG13499, AG05142, and AG14751 and the Alzheimer's Association (C.E.F.) and by NIA grant AG17265 (E.C.). C.E.F. is co-founder, stockholder, and member of the science advisory board of Acumen Pharmaceuticals Inc., which is developing treatments for Alzheimer's disease.

NEED HUMAN SPECIMENS FOR RESEARCH?

The NCI Cooperative Human Tissue Network (CHTN) provides normal, benign, pre-cancerous and cancerous human tissue to the scientific community for basic and developmental studies in many areas of cancer research. Tissue micro-arrays are also available. Contact the CHTN website at: http://www-chtn.ims.nci.nih.gov, or 1-866-GO2-CHTN (1-866-462-2486).

The NCI Cooperative Breast Cancer Tissue Resource (CBCTR) can provide access to formalin-fixed, paraffin-embedded primary breast cancer specimens, with associated pathology and clinical data. The collection is particularly well suited for validation studies of diagnostic and prognostic markers. The CBCTR also has breast cancer tissue micro-arrays designed to provide high power for studies of stage specific markers. Contact the CBCTR website at: http://www-cbctr.ims.nci.nih.gov, or Ms. Sherrill Long, Information Management Services, Inc., (301) 984-3445; e-mail: longs@imsweb.com.

The AIDS and Cancer Specimen Resource (ACSR) provides qualified researchers with tissue, cell, blood and fluid specimens, as well as clinical data from patients with AIDS and cancer. The specimens and clinical data are available for research studies, particularly those that translate basic research findings to clinical application. Contact the ACSR website at: http://acsb.ucsf.edu, or Dr. Jodi Black, (301) 402-6293; e-mail: jb377x@nih.gov.

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The Breast, Ovarian and Colorectal Cancer Family Registries (CFRs) includes two international registries: the Cancer Family Registry for Breast Cancer Studies (Breast CFR) and the Cancer Family Registry for Colorectal Cancer Studies (Colon CFR). The Breast CFR provides family history information, biological specimens and epidemiologic and clinical data from clinic-based and population-based families at risk for breast and ovarian cancers. The Breast CFR infrastructure is particularly suited to support interdisciplinary and translational breast cancer research. Similarly, the Colon CFR collection includes family history information, epidemiologic and clinical data, and related biological specimens from individuals with colorectal cancer and their families. The Colon CFR is a resource for population and clinic-based, translational research in the genetic epidemiology of colorectal cancer. Contact the CFRs website at http:// epi.grants.cancer.gov/CFR/.

The NCI Cooperative Prostate Cancer Tissue Resource (CPCTR) can provide access to over 4,000 cases of formalin-fixed, paraffin-embedded primary prostate cancer specimens, with associated pathology and clinical data. Fresh-frozen tissue is also available with limited clinical follow up information. In addition, slides from prostate cancer tissue micro-arrays with associated pathology and clinical data are now available. Contact the CPCTR website at: http://www.prostatetissues.org, or Mr. Steve Marroulis, Information Management Services, Inc., (301) 680-9770; e-mail: marrouliss@imsweb.com.

The NCI Clinical Trials Cooperative Groups have banked tumor specimens from large numbers of uniformly treated cancer patients with a variety of malignancies. Each group has a review process for research proposals. If proposals receive favorable reviews, specimens with clinical, treatment and outcome data can be made available to researchers through collaborative arrangements. These banked specimens are most useful for clinical correlative studies on uniformly treated patient populations. Contact the NCI Specimen Resource Locator website at: http://www.cancer.gov/specimens, or the NCI Tissue Expediter, e-mail: tissexp@mail.nih.gov; (301) 496-7147.

Each of the resources listed above has an established review process for specimen requests and/or requirements that must be met for access to specimens. Additional details may be obtained from the resource websites and/or resource contacts.

The NCI Specimen Resource Locator is a web-based database to help researchers locate appropriate sources of normal, benign, pre-cancerous and cancerous human tissue specimens for cancer research, http://www.cancer.gov/specimens**.**

Other human specimen resources, including a variety of tissue micro-arrays, are also available. Contact the NCI Tissue Expediter, (301) 496-7147; e-mail: tissexp@mail.nih.gov.

Direct Sub-Angstrom Imaging of a Crystal Lattice

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Achieving sub-angstrom imaging has been a long-standing goal for electron microscopy. Improved resolution allows not only a wider range of materials to be imaged, but also allows each material to be imaged in several possible orientations. It provides much improved sensitivity to atomic arrangements at defects and interfaces, down to the single-atom level for the first time in some instances. Sub-angstrom information transfer can be accomplished in an uncorrected transmission electron microscope (TEM) with tilted illumination (*1*), but the information in the images becomes extremely delocalized and the increased information transfer is only in the direction of the tilt. Underfocusing the main imaging lens can also partially compensate for the existence of spherical aberration (*2*), but the images recorded are similarly highly delocalized and contain low signal strength. Postprocessing of focal-series data can overcome some of these limitations (*3*, *4*). However, this is not direct imaging because it requires the collection of many images, and extensive processing of image data increases the risk of the introduction of image artifacts. Previous evidence of a sub-angstrom point spread function from a scanning TEM (STEM), with apparent spots in a Fourier transform and an intensity profile of a single atom (*5*), is not unambiguous because such measurements are sensitive to errors due to noise, instabilities in the microscope, and incorrect adjustment of the detector black level.

An indisputable test for demonstrating sub-angstrom resolution is a Si crystal observed in the [112] orientation, because it contains pairs of Si columns 78 pm apart. Figure 1, A and B, shows an image recorded with a VG Microscopes HB603U 300-kV STEM fitted with a Nion aberration corrector. It was recorded in the annular dark-field (ADF) imaging mode. In a STEM, electron optics focus a beam of electrons into a narrow spot, or probe, which is scanned over the sample in a raster. The ADF detector's collects electrons scattered by the sample to angles greater than the detector inner radius, which in this case was 0.0009 Gray (90 mrad). Such high-angle scattering is largely incoherent thermal diffuse scattering, which means that the resolution observed in the image is determined by the intensity distribution of the illuminating probe (*2*). Before correction, without a large defocus, the optimum (Scherzer) resolution limit for ADF imaging on this microscope was 0.13 nm. The addition of the corrector allows the probe-forming aperture to be opened up to give an expected probe size in the range of 60 pm. In Fig. 1, A and B, pairs of atomic columns can be resolved with a spacing of 78 pm. The Fourier transform of this image (Fig. 1C) shows clear information transfer to 71 pm, and there is apparent lattice information at 61 pm. We expect this spot to be weak, because at such a high spatial resolution, the physical width of the atoms is substantial and reduces the contrast between closely spaced columns. There is no evidence that the high-frequency spots are due to incorrect background level or distortions (*6*), and the simulated image profile compares well with the experimental data (Fig. 1D).

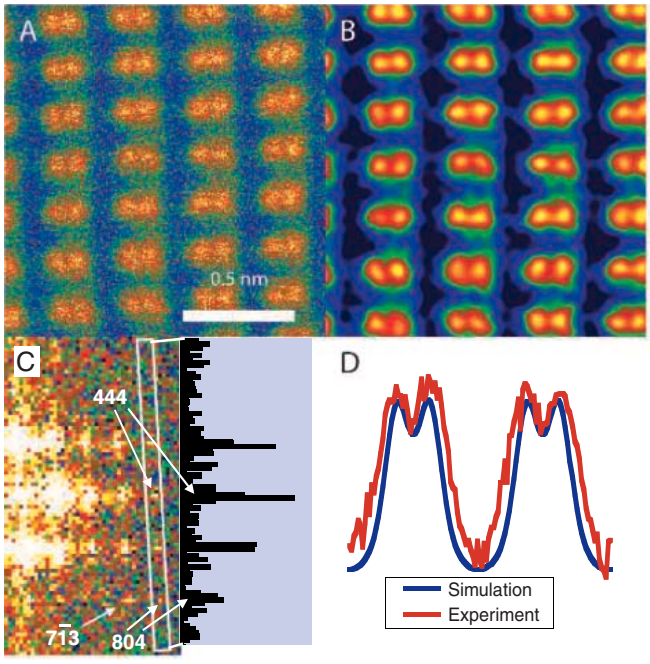

Fig. 1. (**A** and **B**) ADF images of Si[112] recorded with an aberration-corrected STEM. The image in (B) has been low-pass filtered to reduce the noise, and the small effects of image drift during the scan have been unwarped. (**C**) The modulus of the Fourier transform of the image data and a profile through the spots enclosed by the box. The 444 spacing (78 pm) corresponds to the smallest atomic column spacing, and there is information transfer to the 713 (71-pm) spacing and weak transfer at the (804) 61-pm spacing. (**D**) An intensity profile through two column pairs in (A), formed by summing over a width of 10 pixels. A simulated profile (*6*) is shown for comparison.

In summary, we have demonstrated direct sub-angstrom resolution with an aberration-corrected STEM, an advance that allows materials and nanostructures to be imaged with a new level of sensitivity. We expect light columns to be visible in the presence of adjacent heavy columns and individual dopant or impurity atoms to be detectable within materials, at defects and interfaces, and on their surfaces. Such capability should enable a new understanding of the atomic-scale origins of properties with applications in the materials, chemical, and nanosciences.

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6. Materials and Methods are available as supporting material on *Science* online.
7. This research was sponsored by the Laboratory Directed Research and Development Program of Oak Ridge National Laboratory (ORNL), managed by UT-Battelle LLC for the U.S. Department of Energy under contract no. DE-AC05-00OR22725, and by an appointment to the ORNL Postdoctoral Research Program administered jointly by ORNL and the Oak Ridge Institute for Science and Education.

Supporting Online Material
www.sciencemag.org/cgi/content/full/305/5691/1741/DC1
Materials and Methods
Figs. S1 and S2

1 June 2004; accepted 5 August 2004

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Environmentally Induced Foregut Remodeling by PHA-4/FoxA and DAF-12/NHR

Wanyuan Ao,[1]* Jeb Gaudet,[1]*† W. James Kent,[2] Srikanth Muttumu,[1] Susan E. Mango[1]‡

Growth and development of the *Caenorhabditis elegans* foregut (pharynx) depends on coordinated gene expression, mediated by pharynx defective (PHA)-4/FoxA in combination with additional, largely unidentified transcription factors. Here, we used whole genome analysis to establish clusters of genes expressed in different pharyngeal cell types. We created an expectation maximization algorithm to identify cis-regulatory elements that activate expression within the pharyngeal gene clusters. One of these elements mediates the response to environmental conditions within pharyngeal muscles and is recognized by the nuclear hormone receptor (NHR) DAF-12. Our data suggest that PHA-4 and DAF-12 endow the pharynx with transcriptional plasticity to respond to diverse developmental and physiological cues. Our combination of bioinformatics and in vivo analysis has provided a powerful means for genome-wide investigation of transcriptional control.

A critical question in developmental biology is how complex programs of gene expression are orchestrated by a class of regulators known as selector genes. Selector genes code for transcription factors that autonomously govern the fates of groups of cells related to each other by virtue of their cell type, position, or affiliation to an organ (*1*). For example, the FoxA transcription factor PHA-4 dictates the identity of cells within the *Caenorhabditis elegans* pharynx. Embryos that lack *pha-4* fail to generate pharyngeal cells, and conversely ectopic *pha-4* is sufficient to drive nonpharyngeal cells toward a pharyngeal fate (*2*, *3*). PHA-4 also functions during postembryonic development, because reduction of *pha-4* activity at birth is lethal (*4*). These dramatic phenotypes reflect the global regulatory role of PHA-4 within pharyngeal cells. Many, perhaps all, genes selectively expressed within the pharynx are activated directly by PHA-4, including genes expressed in different pharyngeal cell types and at different developmental stages (*4*). This strategy raises the question of how a single transcription factor can mediate diverse transcriptional outcomes within different cellular contexts. Combinatorial regulation by multiple transcription factors is one likely mechanism. However, few transcription factors have been discovered that could act in conjunction with PHA-4 (*5*, *6*). Here, we explore the cis-regulatory logic for expression within pharyngeal cells, with a particular focus on growth control in response to food availability.

Gene expression profiles for pharyngeal cell types. To analyze expression profiles within subsets of pharyngeal cells, we subdivided 339 candidate pharyngeal genes previously identified by microarray analysis (Fig. 1, fig. S1, and table S1) (*4*, *7*). We took advantage of RNA in situ patterns (*8*), reporter expression, and placement on the *C. elegans* Topological Expression Map (*9*) (Topo Map) to generate five clusters of genes with shared expression profiles. On the Topo Map, genes are positioned according to their correlated expression across 553 independent microarray experiments to generate "mountains" that reflect expression within a common cell type or cellular process. The clusters of pharyngeal genes encompassed 65% of the microarray positives and defined genes expressed in pharyngeal muscles (Ph-M), pharyngeal glands (Ph-G), pharyngeal marginal cells (Ph-MC), epithelia (Epi), and pharyngeal muscle and marginal cells combined (Ph-MMC) (fig. S1 and table S1). We validated these assignments with green fluorescent protein (GFP) reporters for previously uncharacterized members of each cluster (Fig. 1).

The compendium of pharyngeal gene clusters provided a tool to explore the regulatory circuitry for cell type–specific

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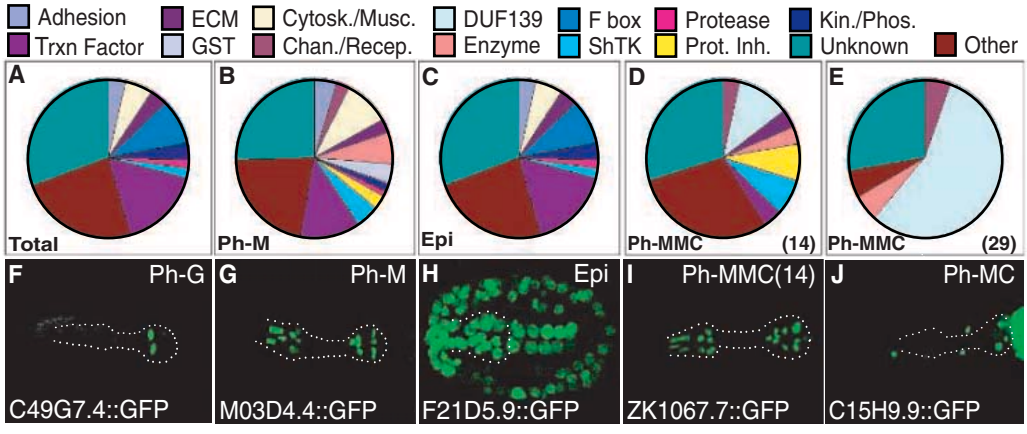

Fig. 1. Five pharyngeal clusters. Pie charts of protein categories for all candidate pharyngeal genes (**A**) and individual clusters (**B** to **E**). GST, glutathione *S*-transferase; ECM, extracellular matrix protein; Prot. Inh., protease inhibitor; ShTK, ShTK domain–containing protein; Chan./Recep., channel molecule or receptor; Cytosk./Musc., cytoskeleton or muscle protein; Enzyme, metabolism enzyme; DUF139, domain of unknown function 139 domain; Trnx Factor, transcription factor; and Kin./Phos., kinase or phosphatase. (**F** to **J**) Previously uncharacterized genes were expressed as predicted. C49G7.4 in Ph-G, confirmed by co-staining with a gland-specific antibody, J126 (*19*), M03D4.4 in Ph-M, F21D5.9 in embryonic Epi, ZK1067.7 in late muscles and marginal cells from Topo Mounts 14 or 29 (Ph-MMC) (*9*), and C15H9.9 in Ph-MC. Dotted lines indicate the pharynx [(F) to (J)].

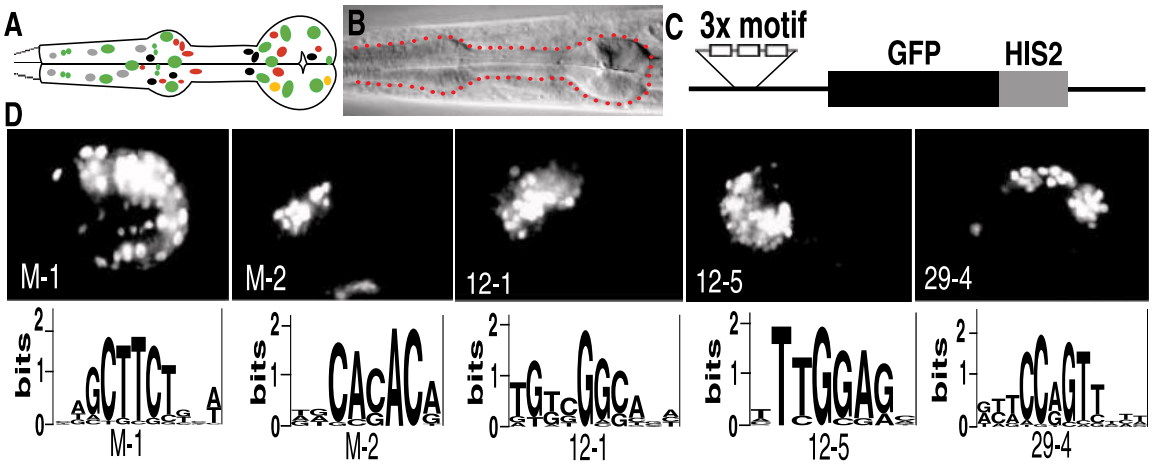

Fig. 2. Predicted cis-regulatory motifs function in vivo. (**A**) Diagram of pharyngeal nuclei (green, myoepithelia; red, neurons; gray, pharyngeal epithelia cells; orange, gland cells; and black, marginal cells). (**B**) Head of an adult worm featuring the bilobed pharynx (dotted). (**C** and **D**) Five of seven motifs displayed enhancer activity in embryos when placed upstream of a minimal *pes-10* reporter (C) (*4*), which alone had no activity (*7*).

expression. We created the Improbizer algorithm (*10*) to identify potential cis-regulatory sites enriched in the pharyngeal gene clusters (*11*). Improbizer searches for motifs in DNA or RNA sequences that occur with unexpected frequency by using a variation of the expectation maximization algorithm (*12*). As a negative control, we examined groups of genes (DNA synthesis or a set of 194 randomly selected genes) that were not specifically expressed in the pharynx and removed motifs common to both the pharyngeal and negative control sets. This analysis identified seven candidate regulatory elements (Fig. 2, fig. S2, and tables S4 and S5). To test whether these elements were biologically active, we introduced three copies of each motif upstream of a minimal *pes-10* promoter (*13*) and examined them for their ability to activate expression.

Five of seven candidate motifs displayed enhancer activity, indicating that our bioinformatics approach efficiently identified cis-regulatory elements that functioned in vivo. Four were active within restricted domains, whereas one, M-1, behaved ubiquitously throughout the embryo (Fig. 2D). Motif M-2, identified from Ph-M, was the most specific and activated expression within pharyngeal muscles. GFP expression initiated during mid-embryogenesis (bean stage) (*14*), peaked during pharyngeal morphogenesis (two- to threefold stages), and decreased dramatically after hatching. Motif 29-4, identified from Ph-MMC, was active in anterior embryonic cells, including those of the pharynx. Reporters were expressed in the head and broadly throughout the pharynx beginning at mid-embryogenesis (1.5- to 2-fold stage). Robust expression was maintained until hatching, after which only a few cells continued to express. Two regulatory motifs, 12-1 and 12-5, were associated with the Epi cluster and activated expression in ectoderm (pharynx, neurons, and epidermis). For example, reporters for 12-5 expressed GFP in the pharynx (71%); pharynx and neurons (24%); or pharynx, neurons, and epidermis (5%). Both 12-1 and 12-5 were active in bean-stage embryos. Reporters for 12-1 maintained GFP expression to adulthood, whereas 12-5-dependent expression peaked at the two- to threefold stage and subsequently declined. These five cis-regulatory elements suggest a simple code for expression within pharyngeal epithelia. We propose that PHA-4 sites contribute to organ-wide activation throughout the pharynx whereas additional elements impart positional (29-4) or cell-type (M-2, 12-1, and 12-5) information.

Table 1. Pharyngeal muscle gene expression is reduced when M-2 or *daf-12* is mutated under growth conditions. GFP expression was determined in independent transgenic lines (*n*), with >20 animals examined per line. "mut" indicates mutant M-2 sites. Inactivation of M-2, *daf-12*, or both lead to an equivalent reduction in GFP expression.

Reporter	Strain	Strong (*n*)	Reduced (*n*)	Dramatically reduced (*n*)	None (*n*)
myo-2P::GFP	Wild type	4	2	3	1
myo-2(mut)P::GFP	Wild type	1	4	8	4
myo-2P::GFP	*daf-12 (m20)*	0	1	3	2
myo-2(mut)P::GFP	*daf-12 (m20)*	0	0	3	3
ceh-22P::GFP	Wild type	2	6	1	2
ceh-22(mut)P::GFP	Wild type	1	1	6	4
ceh-22P::GFP	*daf-12 (m20)*	0	4	8	2
ceh-22(mut)P::GFP	*daf-12 (m20)*	0	2	8	2

The M-2 motif. We examined the activity of the cis-regulatory elements within natural pharyngeal promoters to address their importance for endogenous genes. We focused on the M-2 motif because it was highly selective for pharyngeal muscle. To identify relevant instances of the motif, we chose four genes that were expressed in pharyngeal muscles and carried a perfect match to the M-2 consensus in *C. elegans* and the related species *C. briggsae* (*15*): the heavy chain myosin genes F45G2.2 and *myo-2* (*16*), the Nkx2.5 homeobox homolog *ceh-22* (*17*, *18*), and the predicted zinc finger M03D4.4.

The M-2 motif was required in natural promoters for robust expression. Whereas most transgenic animals expressed GFP strongly in pharyngeal muscles throughout life, expression was reduced dramatically at all stages when M-2 was mutated (Fig. 3D and Table 1) (*19*). Fewer animals had GFP, and expression was weaker for those that did. The presence of additional regulatory elements accounts for the residual expression observed for promoters bearing M-2 mutations (*4*, *17*, *20*).

A current challenge for computational searches for cis-regulatory sites is to identify the trans-acting factors and upstream regulatory pathways that impinge upon these sites. Motifs 12-1 and 12-5 resemble known binding sites for the novel factors PEB-1 and CEB/Pα, repectively (*21*, *22*). We considered that T-box proteins might recognize the M-2 element (*23*); however, no effect on M-2-dependent expression was observed when eight predicted T-box genes were inactivated (table S6). We used a yeast one-hybrid screen (*24*) and obtained a single candidate, isolated 12 times, that recognized six copies of an intact M-2 motif but not a mutated version (Fig. 3B). This candidate was *daf-12*, which encodes

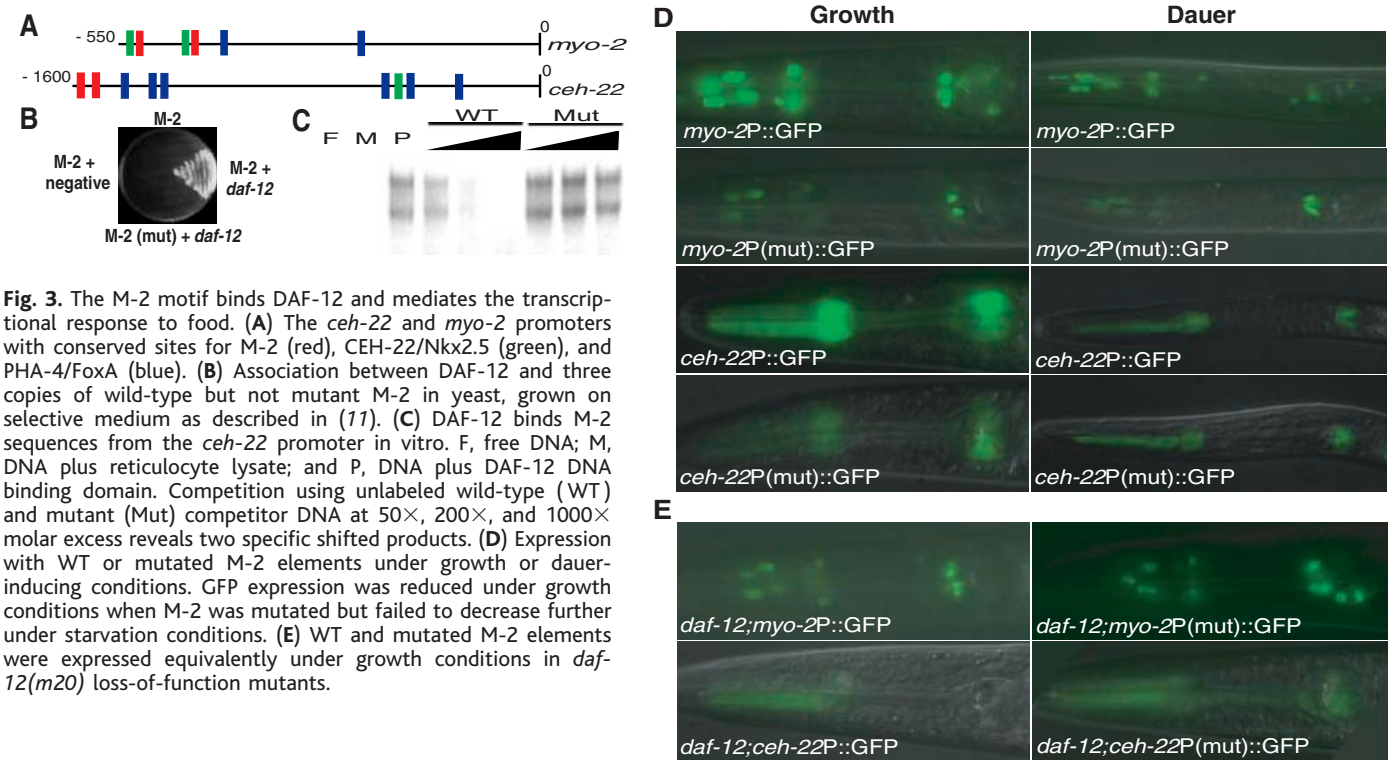

Fig. 3. The M-2 motif binds DAF-12 and mediates the transcriptional response to food. (**A**) The *ceh-22* and *myo-2* promoters with conserved sites for M-2 (red), CEH-22/Nkx2.5 (green), and PHA-4/FoxA (blue). (**B**) Association between DAF-12 and three copies of wild-type but not mutant M-2 in yeast, grown on selective medium as described in (*11*). (**C**) DAF-12 binds M-2 sequences from the *ceh-22* promoter in vitro. F, free DNA; M, DNA plus reticulocyte lysate; and P, DNA plus DAF-12 DNA binding domain. Competition using unlabeled wild-type (WT) and mutant (Mut) competitor DNA at 50×, 200×, and 1000× molar excess reveals two specific shifted products. (**D**) Expression with WT or mutated M-2 elements under growth or dauer-inducing conditions. GFP expression was reduced under growth conditions when M-2 was mutated but failed to decrease further under starvation conditions. (**E**) WT and mutated M-2 elements were expressed equivalently under growth conditions in *daf-12(m20)* loss-of-function mutants.

Table 2. M-2 is required for recovery of pharyngeal muscle gene expression during dauer exit. Entry and exit into the dauer stage were induced by removal or readdition of food, respectively. GFP expression was scored at 0, 3, or 8 hours after food restoration. Entries indicate the number of worms examined.

Reporter	0 hours recovery	3 hours	8 hours
myo-2P::GFP	weak:16 strong: 4	weak:16 strong: 4	weak: 2 strong:18
myo-2(mut)P::GFP	weak:20 strong: 0	weak:20 strong: 0	weak:20 strong: 0
ceh-22P::GFP	weak:14 strong: 6	weak:14 strong: 6	weak: 4 strong:16
ceh-22(mut)P::GFP	weak:20 strong: 0	weak:20 strong: 0	weak:20 strong: 0

a nuclear hormone receptor similar to the pregnane X and vitamin D receptors (*25*, *26*). Electrophoretic mobility shift assays with the *ceh-22* promoter revealed that DAF-12 bound M-2-containing sequences in vitro and that binding was specific (Fig. 3C). These findings demonstrate that the M-2 element is recognized by DAF-12 in vitro.

To ascertain the importance of DAF-12 for M-2 activity in vivo, we examined *myo-2P::GFP* and *ceh-22P::GFP* in *daf-12(m20)* loss-of-function mutants (*27*). Mutant animals expressed GFP at a reduced level: Both the number of cells and GFP intensity were lower, similar to the effects of M-2 mutations (Fig. 3D and Table 1). Animals carrying double mutations in *daf-12* and M-2 resembled those with either single mutation, as expected if DAF-12 binds M-2 (Fig. 3E and Table 1). We conclude that DAF-12 is required for *myo-2* and *ceh-22* expression and functions through the M-2 element.

M-2 and modulation of expression by food availability. DAF-12 is a critical regulator of developmental progression and response to environmental cues (*28*, *29*). When conditions are harsh, nematode larvae enter a long-lived state of diapause called the dauer stage. Dauer larvae exhibit multiple adaptations to stress, including remodeling of the pharynx to become thinner radially and cessation of feeding. The choice between growth and dauer development depends on insulin, transforming growth factor–β, and heterotrimeric guanine nucleotide–binding protein–coupled receptor signaling pathways, which converge on a handful of transcription factors, including DAF-12 (*25*, *26*, *30*). A key question is how DAF-12 executes its biological functions to mediate these developmental events.

Given the role of DAF-12, we tested whether the M-2 motif modulates expression in dauer larvae. Wild-type *myo-2P::GFP* and *ceh-22P::GFP* were each expressed strongly in pharyngeal cells and down-regulated in dauer animals (Fig. 3D and Table 1) (*31*). Once food was restored, GFP gradually returned to its previous robust level as animals exited the dauer program (Table 2). By contrast, animals bearing a mutated M-2 motif failed to decrease expression in dauer larvae or reactivate expression upon feeding (Fig. 3D and Table 2). These effects were specific because transcriptional or translational PHA-4::GFP constructs, which lack an M-2 element, were not modulated in dauer larvae (*19*). We conclude that regulation of *myo-2* and *ceh-22* during dauer development depends critically on the M-2 motif.

Reduced *myo-2* and *ceh-22* expression in dauer larvae could reflect repression by DAF-12 or loss of activation. To distinguish between these possibilities, we examined CEH-22::GFP in *daf-2(insulin receptor); daf-12* double mutants, which form dauer larvae despite reduced *daf-12* (*30*). We reasoned that GFP expression would decrease normally in *daf-12* dauer larvae if down-regulation reflected loss of activation but would increase relative to the wild type if down-regulation required DAF-12 repression. We found that CEH-22::GFP was derepressed in *daf-2; daf-12* double mutants and pharyngeal morphology resem-

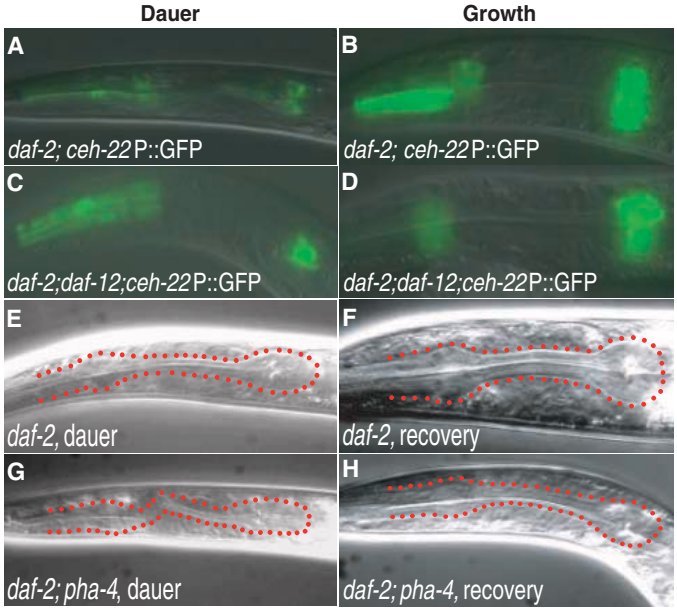

Fig. 4. (**A** to **D**) DAF-12 is required to repress pharyngeal muscle expression in dauer larvae. Reduction of *ceh-22*P::GFP in dauer larvae [(A) and (B)] was lost in *daf-12* mutants [(C) and (D)]. (**E** to **H**) PHA-4 is required for dauer exit but not entry. *daf-2(ts); pha-4(RNAi)* animals were shifted to a nonpermissive temperature to induce dauer entry and pharyngeal remodeling [(E) and (G)]. When transferred to permissive temperature, *daf-2(ts); pha-4(RNAi)* animals failed to exit the dauer stage and resume growth [(F) and (H)].

bled that of growth conditions (Fig. 4). These data suggest that DAF-12 functions as a repressor in dauer larvae and that repression is essential for pharyngeal remodeling.

We identified pharyngeal genes with one or more predicted M-2 motifs and surveyed those genes for regulation during dauer development. A total of 94 pharyngeal genes carried a conserved M-2 element, of which 26 were down-regulated in dauer animals (28%) (*31*) (table S7). Conversely, 191 genes lacked a conserved M-2 element, of which 25 were modulated in dauers (13%). The enrichment of the M-2 element in dauer-regulated genes supports a role for this motif in response to environmental conditions. The majority of dauer-regulated genes were associated with pharyngeal muscle clusters (22 of 26, or 85%) (table S7) and included genes important for feeding, growth, and metabolism. For example, *inx-6* is required for electrical coupling between pharyngeal muscles during feeding (*32*), *nrs-2* and *cyp-17* are predicted to contribute to protein synthesis or folding, and *ech-4* is likely involved in fatty acid metabolism (*33*). These target genes, in addition to *ceh-22*, F45G2.2, and *myo-2*, are attractive candidates to modulate feeding and growth of the pharynx in response to nutritional cues.

Exit from the dauer program, but not entry, was critically dependent on PHA-4/FoxA. Dauer animals were induced by placing *daf-2(ts)* larvae at a nonpermissive temperature. Animals with *pha-4* amounts reduced by RNA interference (RNAi) [*pha-4(RNAi)*] could form dauer larvae as efficiently as *pha-4(+)* animals (Fig. 4). Furthermore, destruction of two predicted Fox binding sites within the *myo-2* promoter did not disrupt dauer-dependent repression of *myo-2*P*::GFP* (fig. S4). We conclude that *pha-4* is not required for the dauer program of development. However, *pha-4* was essential for exit from the dauer program and hypertrophy of the pharynx under growth conditions. When *daf-2(ts)* dauer animals were shifted to permissive temperature for 32 hours, only 13% of *pha-4(RNAi)* larvae recovered and resumed growth ($n = 32$), compared to 92% of *pha-4(+)* animals ($n = 37$). The *pha-4(RNAi)* larvae remained small with partially remodeled pharynges and undeveloped gonads (Fig. 4). *pha-4(RNAi)* dauer larvae were viable for extended periods and resumed growth when *pha-4* activity was restored with food (*19*). Thus, the failure of *pha-4(RNAi)* larvae to reinitiate growth did not reflect a general unhealthiness of these animals. We conclude that the developmental selector gene *pha-4* is redeployed postembryonically to promote larval growth, pharyngeal hypertrophy, and sexual maturation in response to food.

We computationally identified five cis-regulatory sequences and tested their function in vivo. One of these elements mediates the response to environmental cues and is recognized by DAF-12. We speculate that when conditions are favorable, DAF-12, bound to an unidentified ligand, and PHA-4 function as transcriptional activators for genes involved in feeding, hypertrophy, and metabolism. When conditions are harsh, loss of the DAF-12 ligand converts DAF-12 into a transcriptional repressor and thereby triggers dauer development, pharyngeal remodeling, and cessation of feeding. This model provides an explanation for why loss of *daf-12* activity blocks entry into dauer development whereas mutations predicted to interfere with production of a DAF-12 ligand lead to constitutive dauer formation (*34–37*). These studies reveal how the transcriptional program can respond to nutrition to coordinate organ growth and activity.

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38. We thank A. Fire, Y. Kohara, and J. Kiefer for plasmids; R. Barstead for cDNA libraries; K. Yamamoto, H. Mak, and G. Ruvkun for communicating results before publication; and S. Dames for technical assistance. Some nematode strains were provided by the *C. elegans* Genetics Center, funded by NIH National Center for Research Resources. This work was supported by NIH R01-GM56264 to S.E.M. and Canadian Institutes of Health Research funding to J.G. S.E.M. is an associate investigator of the Huntsman Cancer Institute. Molecular interaction data have been deposited in the Biomolecular Interaction Network Database with the accession code 151736. DNA sequencing was supported by the NIH (2P303A42014).

Supporting Online Material

www.sciencemag.org/cgi/content/full/305/5691/1743/DC1
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1 July 2004; accepted 4 August 2004

Mitochondrial Fusion Intermediates Revealed in Vitro

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The events that occur during the fusion of double-membraned mitochondria are unknown. As an essential step toward determining the mechanism of mitochondrial fusion, we have captured this event in vitro. Mitochondrial outer and inner membrane fusion events were separable and mechanistically distinct, but both required guanosine 5′-triphosphate hydrolysis. Homotypic trans interactions of the ancient outer transmembrane guanosine triphosphatase, Fzo1, were required to promote the fusion of mitochondrial outer membranes, whereas electrical potential was also required for fusion of inner membranes. Our conclusions provide fundamental insights into the molecular events driving mitochondrial fusion and advance our understanding of the evolution of mitochondrial fusion in eukaryotic cells.

In eukaryotic cells, homotypic mitochondrial fusion events occur continuously throughout the cell cycle. These fusion events are balanced by mitochondrial fission events, and together they function to create a distributable and responsive mitochondrial compartment (*1*). As a result of their endosymbiotic origin, mitochondria possess two structurally and functionally distinct membranes. During mitochondrial fusion, two pairs of these membranes are coordinately and accurately fused. The mechanism by which these four membranes rapidly fuse into two continuous, yet distinct, bilayers is not understood. Mitochondrial membranes have not been observed to undergo fusion with other endomembranes, and conserved core secretory fusion components, such as soluble *N*-ethylmaleimide–sensitive factor attachment protein receptors, are not required for homotypic mitochondrial fusion. These features suggest that mitochondrial fusion evolved independently from secretory fusion and that the fundamental steps in membrane fusion are mediated in a mechanistically unique manner.

Three proteins in yeast—Ugo1, Mgm1, and Fzo1—are required for mitochondrial fusion in vivo based on cytological assays for mitochondrial morphology and mitochondrial content mixing during yeast mating. Ugo1 is an outer membrane protein, with several predicted transmembrane regions, and is a member of the mitochondrial transport family (*2*, *3*). Fzo1 and Mgm1 are highly conserved large guanosine triphosphatases (GTPases) and are components of the mitochondrial outer and inner membranes, respectively (*4*–*6*). Mgm1 is a dynamin-related GTPase protein (DRP) and is tethered to the inner membrane and localized to the intermembrane space (*6*). Genetic analysis indicates that Mgm1 interacts with itself and that its GTPase domain is required for fusion, suggesting that it functions in mitochondrial fusion in a similar way to other DRPs, as a self-assembling GTPase (*7*, *8*). The Fzo1 GTPase is an integral membrane protein that spans the outer membrane twice; it has a small loop of functionally important amino acids in the intermembrane space, and its N-terminal GTPase domain and C terminus are in the cytosol (*9*). The GTPase domain of Fzo1 is most similar to a family of eubacterial GTPases, suggesting that it is derived from the mitochondrial ancestral prokaryote (*10*). Among eukaryotic GTPases, however, the Fzo1 GTPase domain is most closely related to the DRP family GTPase domain (*11*). Members of the Fzo1 GTPase family also possess, like DRPs, regions predicted to form coiled-coil structures, raising the possibility that they are modified but bone fide members of the DRP family and function in mitochondrial fusion through self-interaction and assembly.

Conserved components that mediate mitochondrial fusion and mitochondrial fission play an important role in the transmission of the cell death response in mammalian cells (*12*–*14*). Mutations in two conserved human fusion proteins, Opa1, the Mgm1 ortholog, and Mfn2, an Fzo1 ortholog, result in two types of neurodegenerative diseases: dominant optic atrophy and Charcot-Marie-Tooth neuropathy, respectively, consistent with a role of these proteins in cell death (*15*–*17*).

Mgm1, Fzo1, and Ugo1 have been shown to interact physically (*7*, *8*, *18*), suggesting that outer and inner membranes share a common fusion apparatus, but the exact role of these components and their interactions in fusion are unknown. Indeed, discrete steps in mitochondrial fusion have not been described, primarily because this event has proven difficult to replicate in vitro.

Mitochondrial fusion in vitro. To understand the molecular events required for mitochondrial fusion, we developed an in vitro cytological assay that measures fusion of outer and inner mitochondrial membranes (*19*). Equivalent amounts of mitochondria containing either matrix-targeted green fluorescent protein (m-GFP) or matrix-targeted Discosoma red fluorescent protein (m-dsRed) were mixed together, concentrated by centrifugation, and resuspended in the presence of cytosolic extract, adenosine 5′-triphosphate (ATP), guanosine 5′-triphosphate (GTP), and an energy regeneration system. Fusion of both the outer and inner membranes was assessed by matrix content mixing, reflected by the colocalization of m-GFP and m-dsRed into single individually resolvable mitochondrial structures in three dimensions (Fig. 1, A and B, and movie S1). Fusion was quantified by determining the number of mitochondria containing both m-GFP and m-dsRed and dividing by the total number of mitochondria analyzed ($n > 200$). In vitro mitochondrial fusion occurred in a time- and temperature-dependent manner, consistently attaining 10 to 15% by 30 min at 22°C (Fig. 1, B and C, and fig. S1A). Because we could only detect m-GFP and m-dsRed content-mixing events, the efficiency of mitochondrial fusion in our assay was substantially higher than 10 to 15%. We also observed successive fusion events at time points greater than 30 min, which indicates that our content-mixing assay was an underestimate of total fusion efficiency. Mitochondrial fusion was highly dependent on exogenous nucleotide triphosphates (NTPs) and an energy regeneration system (Fig. 2) but not dependent on the addition of cytosolic extract, indicating that non–mitochondrial-associated proteins were not required for fusion (fig. S1B).

Three criteria indicate that our matrix content-mixing assay was an accurate reflection of mitochondrial fusion. First, mitochondria containing both m-GFP and m-dsRed were larger in diameter along their long axis than mitochondria containing only GFP or dsRed (Fig. 1D; gray bars represent unfused mitochondria, black bars represent fused mitochondria). Consistently, under conditions where fusion was observed, a population of larger single-labeled m-GFP and m-dsRed also appeared (fig. S2, A and B). Second, the relative fluorescence intensity of GFP and dsRed in fused mitochondria was decreased as compared with that of single-labeled mitochondria, indicating that fluorophores were diluted as a result of an increase in mitochondrial volume from fusion (Fig. 1B; average pixel intensity of fused mitochondria was

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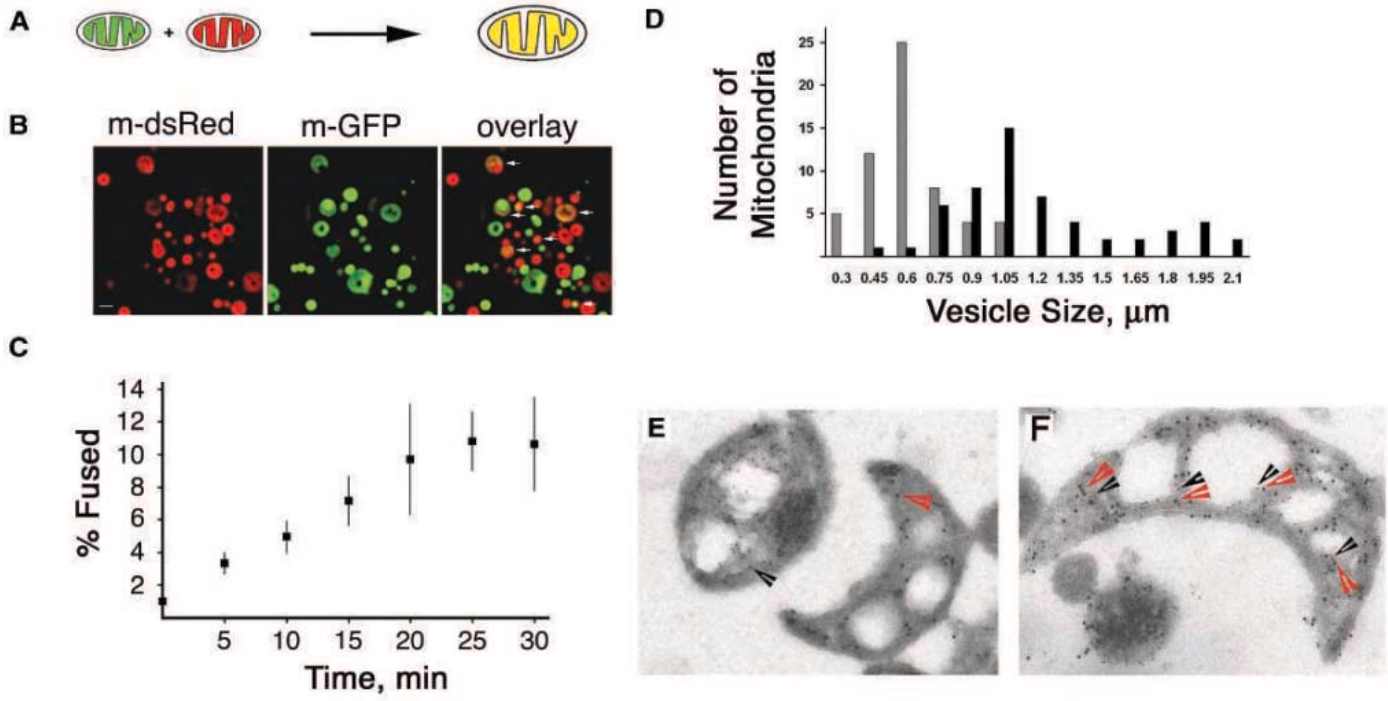

Fig. 1. Mitochondrial fusion in vitro. (**A**) Schematic of in vitro fusion assay. (**B**) Fluorescent images of a fusion reaction with m-GFP and m-dsRed mitochondria. Arrows indicate fused mitochondria. Scale bar, 2 μm. (**C**) Fixed time-point analysis of in vitro mitochondrial fusion. (**D**) Comparison of mitochondrial diameter along long axis of unfused (gray) and fused (black) mitochondria labeled with m-GFP and m-dsRed. Total number of mitochondria analyzed in each case was 56. (**E** and **F**) Immunoelectron microscopy analysis of m-GFP and m-dsRed localization in mitochondria from 0-min (E) and 20-min (F) fusion reactions. Large gold particles (red arrowheads) indicate immunolabeled dsRed and small gold particles (black arrowheads) indicate immunolabeled GFP.

520, average pixel intensity of unfused mitochondria was 1252; a Student's *t* test resulted in $P = 0.000056$). Third, immunoelectron microscopy analysis demonstrated a colocalization of gold-conjugated antibodies directed against GFP and dsRED in fixed mitochondria obtained from a fusion reaction (Fig. 1F). In contrast, in fixed mitochondria obtained from a control reaction in which no fusion was observed, gold-conjugated antibodies directed against GFP and dsRed were observed only in separate mitochondria (Fig. 1E).

Monitoring the behavior of mitochondria labeled with m-GFP and m-dsRed by time-lapse fluorescent microscopy also confirmed that mitochondrial fusion occurred in vitro (movie S2, A and B). Specifically, adjacent mitochondria labeled with m-GFP and m-dsRed were observed to extend toward each other and mix matrix contents. Within our time resolution, membrane extension and matrix content mixing were rapid and unresolved events, similar to in vivo observations in which mitochondrial tubules fuse upon contact (*1*). Thus, the cytological content-mixing assay represents a reliable measure of mitochondrial fusion in vitro.

The energetics of mitochondrial fusion. Two mitochondrial membrane proteins, Fzo1 and Mgm1, required for mitochondrial fusion in vivo, are predicted to be GTPases, and mutations in the GTPase domains of both proteins result in mitochondrial fusion defects in vivo (*4*, *7*, *8*). However, a direct role for GTP hydrolysis in mitochondrial fusion has not been demonstrated.

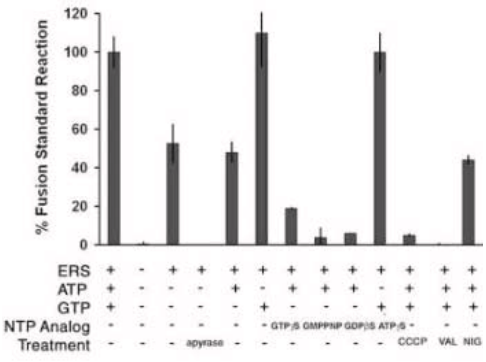

Fig. 2. Mitochondrial fusion in vitro requires GTP hydrolysis and inner mitochondrial membrane potential. In vitro fusion reactions were conducted as described and fusion efficiency is expressed as a percentage of the standard reaction, which contains an energy regeneration system (ERS), 1 mM ATP, and 0.5 mM GTP (*19*). Stage 1 mitochondria were resuspended and placed under stage 2 conditions in the presence of nonhydrolyzable NTP analogs and treatments as described (*19*). Error bars show mean + SD. VAL, valinomycin; NIG, nigericin.

We examined the nucleotide requirements and the effects of nonhydrolyzable nucleotide analogs on content mixing in vitro (Fig. 2). Mitochondrial fusion was completely dependent on NTPs. The energy regeneration system alone supported a small amount of fusion, indicating that isolated mitochondria contain an endogenous pool of NTPs. Treatment of mitochondria with apyrase, added before the assay to deplete NTPs, completely abolished fusion in a reaction containing an energy regeneration system (*20*). The addition of exogenous ATP to a reaction containing the energy regeneration system did not further stimulate fusion, indicating either that mitochondria possess sufficient endogenous ATP to support fusion or that ATP was not required for mitochondrial fusion. In contrast, the addition of exogenous GTP in the presence of the energy regeneration system substantially stimulated the efficiency of mitochondrial fusion, suggesting that the amount of GTP in mitochondria was limiting and required for mitochondrial fusion.

To test whether mitochondrial fusion in vitro required GTP hydrolysis, we added excess nonhydrolyzable GTP analogs to a reaction containing an energy regeneration system and ATP. In the presence of guanosine 5′-*O*-(3′-thiotriphosphate) (GTP-γ-S) or guanosine 5′-monophosphate guanosine 5′ [β,γ-imido]triphosphate (GMPPNP), fusion was substantially reduced,

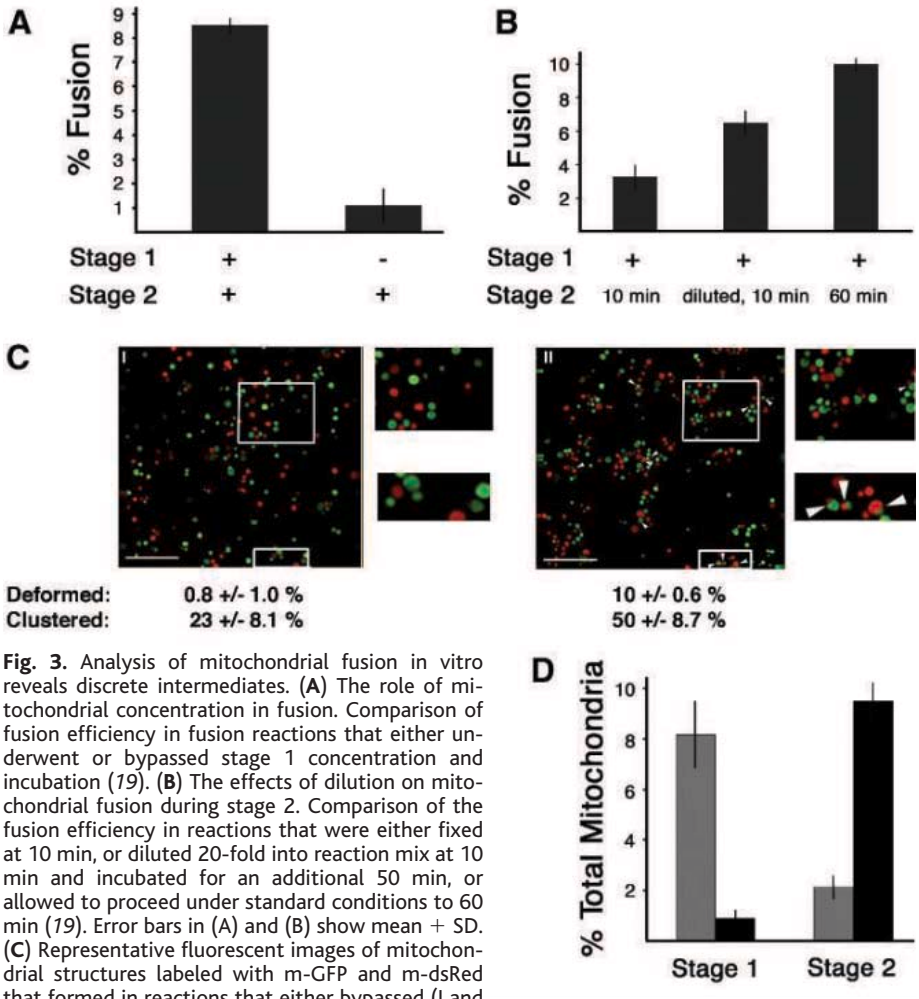

Fig. 3. Analysis of mitochondrial fusion in vitro reveals discrete intermediates. (**A**) The role of mitochondrial concentration in fusion. Comparison of fusion efficiency in fusion reactions that either underwent or bypassed stage 1 concentration and incubation (*19*). (**B**) The effects of dilution on mitochondrial fusion during stage 2. Comparison of the fusion efficiency in reactions that were either fixed at 10 min, or diluted 20-fold into reaction mix at 10 min and incubated for an additional 50 min, or allowed to proceed under standard conditions to 60 min (*19*). Error bars in (A) and (B) show mean + SD. (**C**) Representative fluorescent images of mitochondrial structures labeled with m-GFP and m-dsRed that formed in reactions that either bypassed (I and insets), or underwent stage 1 conditions (II and insets). Scale bar, 8 μm. Quantification of deformed or clustered structures in the total mitochondrial population is listed beneath respective conditions. (**D**) Quantification of deformed mitochondria (gray) and fused mitochondria (black) plotted as a percentage of total mitochondria after stage 1 and after stage 2 ($n > 250$ per condition). Error bars show mean ± SD (gray) and mean + SD (black).

indicating that GTP hydrolysis was essential. The addition of excess guanosine 5′-*O*-(2′-thiodiphosphate) (GDP-β-S) also inhibited fusion, providing further evidence that GTP binding and hydrolysis were essential. In contrast, the addition of an excess amount of nonhydrolyzable ATP [adenosine 5′-*O*-(3-thiotriphosphate)] did not reduce fusion, indicating that in vitro ATP hydrolysis was not required. Thus, GTP but not ATP hydrolysis was required for fusion, consistent with essential roles of the two large GTPases Fzo1 and Mgm1 in mitochondrial fusion in vivo.

During the development of the in vitro assay, we observed that mitochondrial fusion was strongly influenced by yeast cell culture media, particularly the carbon source (fig. S1D). Specifically, mitochondria isolated from cells grown on nonfermentable carbon sources efficiently fused in vitro, whereas those isolated from cells grown on a fermentable carbon source were unable to fuse in vitro. This led us to test directly whether mitochondrial membrane potential played a role in mitochondrial fusion by determining the effects of the electron transport chain uncoupler, carbonylcyanide m-chlorophenylhydrazone (CCCP) (Fig. 2). When CCCP was present during the assay, fusion was abolished. CCCP also inhibits mitochondrial fusion in mammalian cells, indicating that membrane potential is a conserved requirement (*21*).

To dissect the requirement for mitochondrial inner membrane potential, we examined the effects of two additional ionophores on fusion: valinomycin, an electrogenic K^+ ionophore, which specifically dissipates the electrical gradient, and nigericin, an electroneutral K^+/H^+ ionophore, which specifically dissipates the proton gradient (Fig. 2). Mitochondrial content mixing was highly sensitive to valinomycin, but significantly less sensitive to nigericin, indicating that the electrical and not the proton gradient was required for efficient mitochondrial fusion.

Mitochondrial fusion proceeds through discrete and sequential mitochondrial outer and inner membrane fusion events. The in vitro fusion reaction was divided into two experimental stages: Stage 1 included mitochondrial centrifugation, incubation, and resuspension, and stage 2 included the addition of exogenous NTPs and an energy regeneration system (*19*). To assess quantitatively the requirement of stage 1 for fusion efficiency, either mitochondria were centrifuged, incubated, and resuspended in the presence of the energy regeneration system and NTPs, or they were immediately resuspended with the energy regeneration system and NTPs. Content-mixing efficiency in each reaction was determined after 60 min of incubation at 22°C. In the absence of stage 1 treatment, mitochondrial fusion was markedly reduced (Fig. 3A). Both the centrifugation and incubation steps of stage 1 were essential for efficient fusion during stage 2 (fig. S1C). Thus, bringing mitochondrial membranes into close proximity for a critical duration was a required and rate-limiting step. In vivo, this step is likely to be mediated by cytoskeletal elements, such as actin, which in yeast function both to tether and to move mitochondria (*22*).

We then assessed whether mitochondrial concentration was a critical parameter for stage 2 of the reaction (Fig. 3B). After 10 min at stage 2, mitochondria were diluted 20-fold, but the concentrations of the energy regeneration system and NTPs were maintained. The diluted reaction was incubated for an additional 50 min for a total of 60 min. The fusion efficiency of the diluted reaction was reduced, compared with the undiluted 60-min control reaction, indicating that mitochondrial concentration was important, but not essential, during stage 2. In addition, the fusion efficiency of the diluted reaction at 60 min was greater than that of the undiluted control reaction assessed at 10 min, the time of dilution, indicating the existence of dilution-resistant intermediates capable of completing fusion. Thus, during stage 1, intermediate fusion structures formed and went on to fuse in an energy-dependent manner during stage 2.

We examined stage 1 mitochondria with the use of fluorescence microscopy to look for intermediate structures. Two classes of morphological structures were observed: clustered structures with a region lacking a fluorescence signal between adjacent mitochondria (Fig. 3CII) and tightly juxtaposed deformed structures with no region lacking a fluorescence signal between them (Fig. 3CII, arrows). In contrast, mitochondria that bypassed stage 1 and were placed directly under stage 2 conditions for 60 min at 22°C formed a significantly reduced number of both types of structures (Fig. 3CI). Thus, the clustered and deformed structures appear to represent the dilution-resistant fusion intermediates detected during stage 2 of the reaction (Fig. 3B). A comparison of stage 1 mitochondria with stage 2 mitochondria showed significantly fewer deformed stage 2 mitochondria and a proportionally greater number of fused mitochondria, indi-

cating that these structures were productive intermediates in the fusion pathway (Fig. 3D; gray bars represent deformed mitochondria, black bars represent fused mitochondria). There was less of a difference in the number of clustered stage 1 versus stage 2 mitochondria, suggesting that deformed structures represent a more advanced fusion intermediate.

To determine the behavior of outer and inner mitochondrial membranes in these fusion intermediates, we examined stage 1 mitochondria by electron microscopy (EM). EM analysis also revealed at least two classes of mitochondrial structures (Fig. 4A): tightly associated mitochondria without obvious deformation (Fig. 4A, black arrows) and tightly associated and reciprocally deformed mitochondrial pairs (Fig. 4A, red arrows, and Table 1). Higher magnification of deformed mitochondria revealed that they possessed a continuous outer membrane encapsulating a boundary of two separate but tightly aligned and associated inner membranes, suggesting that they were a product of outer membrane fusion in the absence of inner membrane fusion (Fig. 4B, arrows). These structures probably represent the deformed structures observed by fluorescence microscopy (Fig. 4AII). EM analysis revealed that a greater number of deformed structures was observed in stage 1 reactions compared with that of either freshly isolated or stage 2 mitochondria (Table 1 shows stage 1; 27% of stage 1 found in freshly isolated mitochondria, $n > 500$, and 28% of stage 1 found in stage 2 mitochondria, $n > 200$). We also observed by EM a population of tightly associated nondeformed mitochondrial pairs (Fig. 4A, black arrows). In most cases, we were unable to visualize all four mitochondrial membranes within these pairs and thus were unable to determine whether outer membrane fusion had occurred. However, it is likely that these tightly associated pairs of mitochondria represented the clustered structures visualized by fluorescence microscopy (Fig. 4AI).

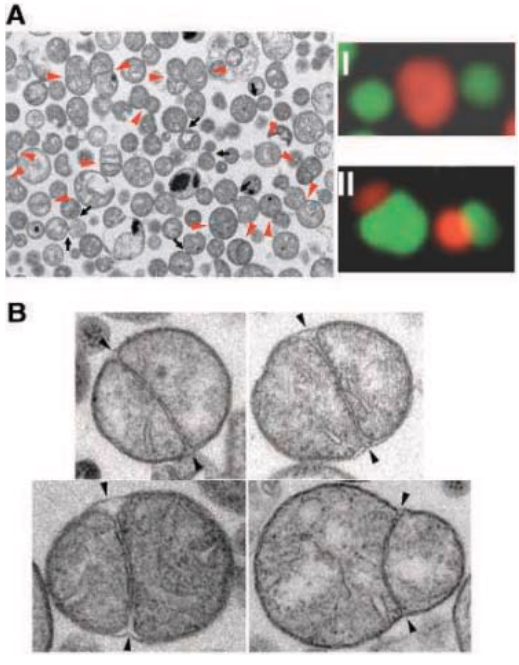

Fig. 4. Fusion of the outer mitochondrial membrane and inner mitochondrial membrane are separable events. (**A**) EM analysis of stage 1 mitochondria. Clustered and deformed mitochondria are indicated by black arrows and red arrowheads, respectively. Magnified fluorescent images of stage 1 mitochondria from Fig. 3C are shown representing clustered (I) and deformed (II) mitochondrial structures. (**B**) EM images of representative deformed mitochondrial pairs. Arrowheads indicate regions of continuous outer membrane encapsulating two separate, but tightly aligned and opposing inner membranes.

To substantiate our EM observations, we developed a fluorescence microscopy–based assay for outer membrane fusion (Fig. 5A) (*19*). Mitochondria labeled with m-dsRED and an outer membrane–targeted GFP (om-GFP) were mixed under various reaction conditions with an equivalent amount of mitochondria labeled with matrix-targeted blue fluorescent protein (m-BFP). If outer membrane fusion occurred in the absence of inner membrane fusion, an outer membrane labeled with om-GFP would be observed surrounding doubly labeled but nonoverlapping matrix compartments labeled with dsRED and BFP (Fig. 5A). Consistent with our EM observations, analysis of stage 1 mitochondria with the use of this assay revealed a significant population of such structures (Fig. 5B, movie S3, and Table 1). In stage 2 reactions, the number of structures in which only the outer membrane had fused was significantly less than the number in stage 1 reactions, confirming that these are intermediates in the fusion pathway (Table 1 shows stage 1; 62% of stage 1 found in stage 2 reactions, $n > 300$). Consistent with our previous observations, outer membranes labeled with om-GFP surrounding colocalized m-dsRed and m-BFP specifically appeared during stage 2, indicating that inner membrane fusion had occurred under these conditions (Fig. 5C).

Using this fluorescence-based assay, we asked whether outer membrane fusion also required GTP and/or inner membrane potential (Fig. 5D). Pretreatment of mitochondria with apyrase severely inhibited outer membrane fusion of mitochondria during stage 1 (*20*). Similarly, the addition of GTP-γ-S, GMPPNP, or GDP-β-S during stage 1 significantly reduced the efficiency of outer membrane fusion, indicating that endogenous GTP was required to promote the fusion of the outer membrane. When CCCP was present during stage 1, outer

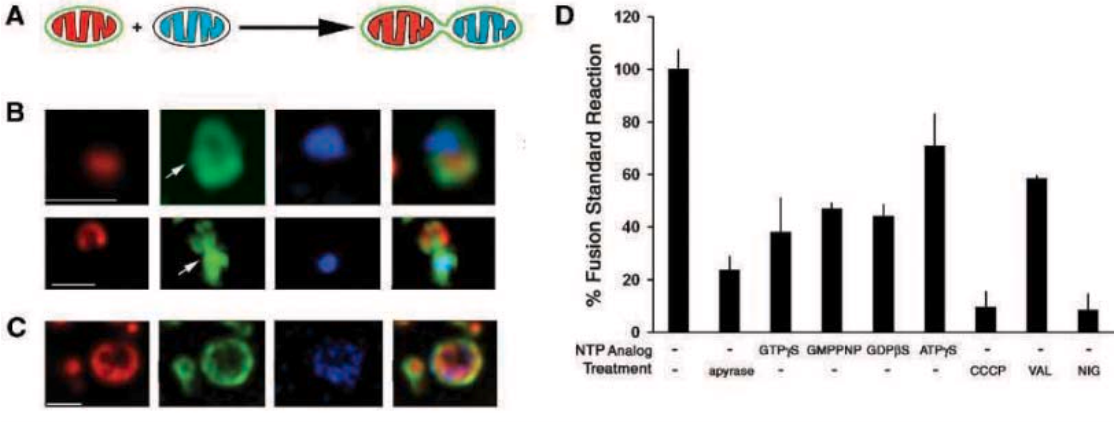

Fig. 5. Mitochondrial outer membrane fusion in vitro. (**A**) Schematic of in vitro outer membrane fusion assay. (**B** and **C**) Fluorescent images of products from fusion reactions with om-GFP/m-dsRed and m-BFP mitochondria after (B) stage 1 (two examples are shown) and (C) stage 2. White arrows in (B) indicate regions of continuous outer membrane connecting two distinct matrix compartments. Scale bar, 1 μm. (**D**) Energetics of outer membrane fusion. In vitro outer membrane fusion reactions were conducted as described (*19*) and fusion efficiency is expressed as a percentage of the standard reaction.

membrane fusion was abolished. Mitochondrial outer membrane fusion was highly sensitive to nigericin but significantly less sensitive to valinomycin, indicating that proton gradient was required for efficient mitochondrial outer membrane fusion.

Our data indicate that outer and inner membrane fusion events were separable and mechanistically distinct. Outer membrane fusion required mitochondrial concentration, was driven energetically by a relatively low (endogenous) concentration of GTP and was dependent on the inner membrane proton gradient (Fig. 5D). Inner membrane fusion, in contrast, required the hydrolysis of a relatively high concentration of GTP and the inner membrane electrical gradient (Fig. 2).

Fzo1 is required for outer membrane fusion and influences the efficiency of inner membrane fusion. Dissection of mitochondrial fusion into separate outer and inner membrane fusion events provides an experimental framework for determining the exact functions of the fusion proteins. Given our fundamental knowledge of membrane fusion events, it seems likely that outer and inner membrane fusion are products of active mechanisms resulting from trans interactions between fusion components on opposing mitochondria (*23*). The ancient and highly conserved transmembrane GTPase, Fzo1, is an excellent candidate for mediating outer membrane fusion.

To assess whether Fzo1 was required during fusion in trans, both heterozygous and homozygous *fzo1* mitochondrial mutant reactions were analyzed. In both heterozygous and homozygous Δ*fzo1* reactions, both outer and inner membrane fusion was completely abolished. However, these defects may have been the result of indirect phenotypes associated with Δ*fzo1* cells. Specifically, the loss of Fzo1 function in cells secondarily causes a loss of mitochondrial DNA and consequently greatly reduces mitochondrial membrane potential, which is essential for mitochondrial fusion (*4*) (Fig. 2). To assess more directly the role of Fzo1 in fusion, we used the hypomorphic, recessive, temperature-sensitive *fzo1-1* allele. Both mitochondrial tubules and mitochondrial DNA in yeast cells harboring the hypomorphic *fzo1-1* allele can be maintained at permissive temperature, but after shifting to nonpermissive temperature, mitochondria rapidly fragment as a result of a block in mitochondrial fusion (*4*).

To analyze *fzo1-1* reactions, both EM analysis and the fluorescence-based assays for mitochondrial outer and inner membrane fusion were used (Table 1). Wild-type mitochondria exposed to nonpermissive temperatures before or during some of the experimental steps in stage 1 were unable to fuse. Thus, to analyze the role of Fzo1, the concentration and incubation steps were performed at permissive temperature and mitochondria were placed at a nonpermissive temperature after resuspension in reactions lacking the energy regeneration system and NTPs (for stage 1) or containing the energy regeneration system and NTPs (for stage 2) and incubated for 60 min.

In homozygous *fzo1-1* stage 1 reactions, mitochondrial outer membrane fusion was significantly compromised under both permissive and nonpermissive conditions, compared with that of the wild type, as assessed by both fluorescence and EM analysis (Table 1). It is likely that Fzo1-1 protein retained partial function in vitro under nonpermissive conditions, explaining why outer membrane fusion was not completely abolished. Significantly, mitochondrial outer membrane fusion was equally compromised in heterozygous *fzo1-1* stage 1 reactions under nonpermissive conditions as compared with that of the wild type. These findings indicate that Fzo1-Fzo1 interactions on opposing mitochondrial membranes were required to promote outer membrane fusion.

We also asked whether Fzo1 function was required for inner membrane fusion by assaying matrix content mixing in homozygous and heterozygous *fzo1-1* reactions under stage 2 conditions (Table 1). At permissive temperature, mitochondrial outer membrane fusion was affected to a greater extent than inner membrane fusion, indicating that the relatively high NTP concentration present during stage 2 partially suppressed the outer membrane fusion defect observed in stage 1 *fzo1-1* reactions. At nonpermissive temperature, both mitochondrial inner and outer membrane fusion were severely compromised by the loss of Fzo1 function in heterozygous and homozygous reactions, as compared with the inner and outer membrane fusion of the wild type. Defects in outer and inner membrane fusion were suppressed in *fzo1-1* mitochondria isolated from *fzo1-1* cells expressing wild-type Fzo1, indicating that they were the result of compromised Fzo1 function. The severe defect in inner membrane fusion observed in *fzo1-1* mitochondria at nonpermissive temperature suggests that Fzo1 trans interactions in the outer membrane were also required for inner membrane fusion.

A model for mitochondrial fusion. Mitochondrial fusion is accomplished through sequential mitochondrial outer and inner membrane fusion events that are mediated by separate and mechanistically distinct machineries. Outer membrane fusion requires GTP and trans Fzo1 interactions on opposing mitochondria, suggesting that GTP promotes outer membrane fusion by means of Fzo1. The conserved Fzo1 cytosolic C-terminal domain forms an intermolecular dimeric antiparallel coiled-coil structure (*24*), suggesting a mechanism for bringing opposing outer membranes into close proximity. The only known fusion protein associated with the inner membrane is the DRP Mgm1. Thus, it is probable that additional components will be required in the inner membrane to mediate the critical steps of membrane association and fusion pore formation.

Although outer and inner membrane fusion can be uncoupled, interactions between Fzo1 and inner membrane fusion proteins, such as Mgm1, play important roles in inner membrane fusion. Biochemical interactions between Fzo1

Table 1. Mitochondrial outer and inner membrane fusion of *fzo1-1* mitochondria relative to that of the wild-type (WT) control. When fluorescence was used, outer membrane fusion was quantified after stage 1 by determining the number of mitochondria with an om-GFP–labeled outer membrane surrounding doubly labeled but nonoverlapping dsRED- and BFP-labeled matrix compartments and dividing by the total number of mitochondria analyzed. When EM was used, outer membrane fusion was quantified after stage 1 by determining the number of mitochondrial pairs that were reciprocally deformed and surrounded by a continuous outer membrane. A significant number of these structures was observed in input mitochondria used in the fusion assays (27% of wild type, $n > 500$). Inner membrane fusion was quantified after stage 2 by determining the number of mitochondria labeled with overlapping dsRED- and GFP-labeled matrix compartments and dividing by the total number of mitochondria analyzed. Absolute percentages are indicated in parentheses for wild-type reactions for each assay and condition. Temp., temperature.

Type of mitochondria	Temp. (°C)	Outer membrane fusion		Inner membrane fusion
		Fluorescence (%)	EM (%)	Fluorescence (%)
WT × WT	22°	100 (9.2%, $n > 600$)	100 (6.3%, $n > 300$)	100 (12%, $n > 300$)
WT × WT	37°	100 (8.8%, $n > 400$)	100 (6.4%, $n > 300$)	100 (11.8%, $n > 400$)
fzo1-1 × *fzo1-1*	22°	13 ($n > 600$)	64 ($n > 800$)	88 ($n > 300$)
fzo1-1 × *fzo1-1*	37°	14.5 ($n > 400$)	41 ($n > 600$)	5.5 ($n > 200$)
fzo1-1 × WT	22°	16 ($n > 600$)	60 ($n > 300$)	92 ($n > 200$)
fzo1-1 × WT	37°	16 ($n > 400$)	52 ($n > 300$)	24 ($n > 300$)

and Mgm1 have been demonstrated and involve the outer membrane fusion protein Ugo1 (*7*, *8*). The exact nature of the interactions between Fzo1, Ugo1, and Mgm1 and their specific roles in mitochondrial fusion remain largely unknown. However, Ugo1 functions as an adaptor between Fzo1 and Mgm1 (*18*). Fzo1 interactions with inner membrane components may be required in a mechanical manner for the formation of regions of close inner and outer membrane contact within mitochondria. Such regions of contact would function to bring inner membranes into closer proximity after outer membrane fusion and also perhaps to eliminate cristae in the vicinity of fusion. Indeed, by EM analysis, no cristae are observed at sites of inner membrane contact (Fig. 4B). Alternatively, but not exclusively, Fzo1 may function in a regulatory manner by stimulating, by means of GTP cycle–dependent conformations, events in the inner membrane required for fusion.

Fzo1 is a key player in the evolution of mitochondrial fusion. Based on a phylogenetic analysis, Fzo1 is derived from the eubacterial endosymbiotic precursor of mitochondria (*10*, *11*). Our data showing that Fzo1 plays essential and fundamental roles in the fusion of both outer and inner membranes are consistent with this idea. Phylogenetic analysis of Fzo1 also identifies it as a member of the dynamin-related GTPase family (*11*). The similarity of Fzo1 to DRPs suggests the intriguing possibility that DRPs evolved from a eubacterial progenitor, and that Fzo1, like DRPs, functions to remodel membranes through self-interaction and assembly. An additional evolutionary connection between DRPs and endosymbiotic organelles is that their division also has evolved to require the action of a DRP (*25*).

DRPs most commonly have been shown to function in membrane fission events, such as mitochondrial and chloroplast division and endocytosis (*26*). However, the actions of two DRPs, Fzo1 on the outer membrane and Mgm1 on the inner membrane, are required for mitochondrial membrane fusion. In a fusion event, Fzo1 and Mgm1 may possess modified activities and function through self-assembly only to tubulate, and not divide, regions of outer and inner membrane, thereby creating a bending stress, which can be harnessed for membrane fusion. The utilization of DRPs to drive membrane fusion events mechanistically distinguishes mitochondrial fusion from other fusion events in eukaryotic cells. Understanding their exact mode of action will enhance our understanding of the fundamental principles that underlie membrane fusion events.

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27. We thank F. McNally, M. Paddy, T. Powers, S. Scott, and S. Theg for helpful discussions and comments on the manuscript and A. Cassidy-Stone for experimental assistance. Supported by grants from the NSF (MCB-0110899) and NIH (R01-GM62942A) to J.N.

Supporting Online Material

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Materials and Methods
Figs. S1 and S2
References
Movies S1 to S3

24 May 2004; accepted 23 July 2004
Published online 5 August 2004;
10.1126/science.1100612
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REPORTS

Two-Step Synthesis of Carbohydrates by Selective Aldol Reactions

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Studies of carbohydrates have been hampered by the lack of chemical strategies for the expeditious construction and coupling of differentially protected monosaccharides. Here, a synthetic route based on aldol coupling of three aldehydes is presented for the de novo production of polyol differentiated hexoses in only two chemical steps. The dimerization of α-oxyaldehydes, catalyzed by L-proline, is then followed by a tandem Mukaiyama aldol addition-cyclization step catalyzed by a Lewis acid. Differentially protected glucose, allose, and mannose stereoisomers can each be selected, in high yield and stereochemical purity, simply by changing the solvent and Lewis acid used. The reaction sequence also efficiently produces ^{13}C-labeled analogs, as well as structural variants such as 2-amino– and 2-thio–substituted derivatives.

Hexose carbohydrates play vital roles in biological processes as diverse as signal transduction, cognition, and the immune response. However, the study of this fundamental class of bioarchitecture has been hindered by the paucity of chemical methods for the efficient synthesis and coupling of hexose systems to form polysaccharides and other derivatives (*1*). Specifically, the challenge in selectively linking and functionalizing these monosaccharides lies in distinguishing among their five chemically similar hydroxyl groups. During the last century, chemists have focused on using iterative alcohol protection-deprotection strategies, an approach that typically requires 8 to 14 chemical steps (*1*, *2*). While the abundant and inexpensive supply of native carbohydrates may render such a strategy intuitively attractive, we felt that a de novo enantioselective synthesis of differentially protected hexoses might provide a more efficient approach (*3*–*10*). The appeal of this strategy is that fragments of the hexose can be independently derivatized (isotopically or

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functionally) before assembly of the molecule; thus, there is no longer a need to chemically discriminate between similar groups on a hexose framework.

In this context, the aldol reaction has been applied to the synthesis of carbohydrates on a few occasions; however, the need for iterative oxidation-state adjustments has thus far precluded a broadly used or step-efficient protocol. From a conceptual standpoint, a two-step carbohydrate synthesis can be envisioned based on an iterative aldol sequence using simple α-oxyaldehydes. While the approach is attractive in theory, the practical execution of this synthetic strategy requires two unproven aldol applications: (i) an enantioselective aldol union of α-oxyaldehyde substrates (Aldol Step 1, Fig. 1A) and (ii) a diastereoselective aldol coupling between tri-oxy–substituted butanals and an α-oxyaldehyde enolate (Aldol Step 2, Fig. 1B). In this report, we describe the successful development of both such aldol reactions for the two-step synthesis of enantioenriched, polyol-differentiated hexoses.

The first step in our synthetic scheme (Aldol Step 1) is a stereoselective α-oxyaldehyde dimerization. Beyond the traditional demands of enantio- and diastereocontrol, the reaction requires that the α-oxyaldehyde reagent **1** participate as both a nucleophile and an electrophile, whereas the product **2**, also an α-oxyaldehyde, must not perform as either (Fig. 1A). Recently, we disclosed an organocatalytic strategy that uses L-proline for the regio-, diastereo-, and enantioselective aldol cross-coupling of α-alkyl–bearing aldehydes (*11*–*16*). Notably, the aldehyde-containing products of this reaction do not participate in further aldol chemistry. With this in mind, we attempted to extend this methodology to the coupling of α-oxygenated aldehydes (Fig. 1A).

As shown in Fig. 2, the proline catalyzed α-oxy aldol (Step 1 results) does provide direct and enantioselective access to differentially protected *anti*-1,2 triols. Specifically, exposure of α-triisopropylsilyloxy-acetaldehyde to 10 mol% L-proline at room temperature readily provides enantioenriched [95% enantiomeric excess (ee)] α,γ-oxy–protected L-erythrose **3**, whereas the corresponding reaction of α-benzyloxyacetaldehyde leads to the dimeric aldol adduct **4** in 98% ee. As required, the α-oxyaldehyde products of this new aldol protocol are inert to further proline-catalyzed enolization or enamine addition. We recently published a report outlining the scope and limitations of this α-oxyaldehyde dimerization (*17*).

Having succeeded in our first step, we next focused on adding the third aldehyde building block and achieving cyclization. Given that β-hydroxy aldehydes (the products of Step 1) are relatively inert to enamine addition, we focused instead upon Lewis acid activation for the second aldol coupling. Specifically, we reasoned that a Mukaiyama aldol reaction between an α-oxy-enolsilane **5** and a trioxy-aldehyde **2** (the product of Aldol Step 1) might generate a hexose-oxocarbenium intermediate **6** that would rapidly undergo cyclization to form the carbohydrate ring system (Fig. 1B). This tandem aldol addition and cyclization presents two selectivity issues: (i) The chemoselective preference for the oxocarbenium **6** to undergo cyclization in lieu of further aldol addition and (ii) the diastereoselective construction of two new oxy-stereocenters in the carbon-carbon bond–forming step, which ultimately defines the extent to which one carbohydrate isomer is generated in preference to another (e.g., allose versus altrose versus glucose versus mannose; see Fig. 2).

We first examined the use of the triisopropylsilyl (TIPS)-protected β-oxy aldehyde **3** (Aldol Step 1 product) and the α-acetoxy-

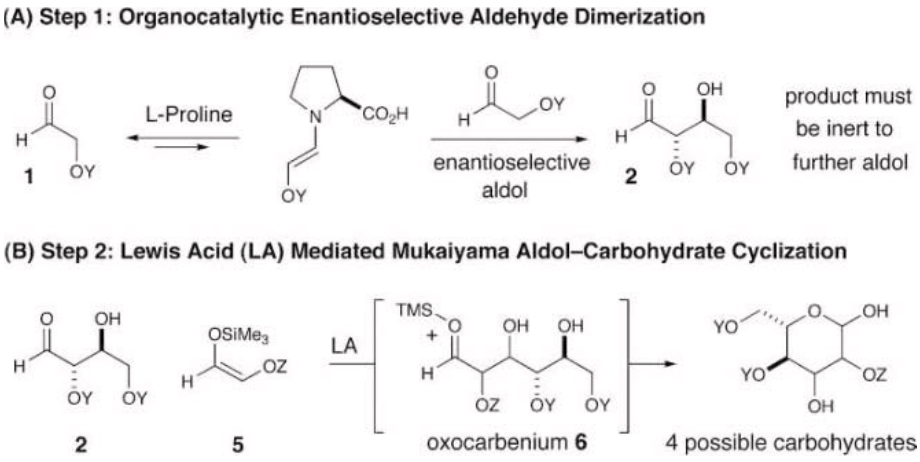


Fig. 1. (**A**) Step 1: Proline-catalyzed enantioselective dimerization of α-oxyaldehydes. (**B**) Step 2: Mukaiyama aldol-carbohydrate cyclization.

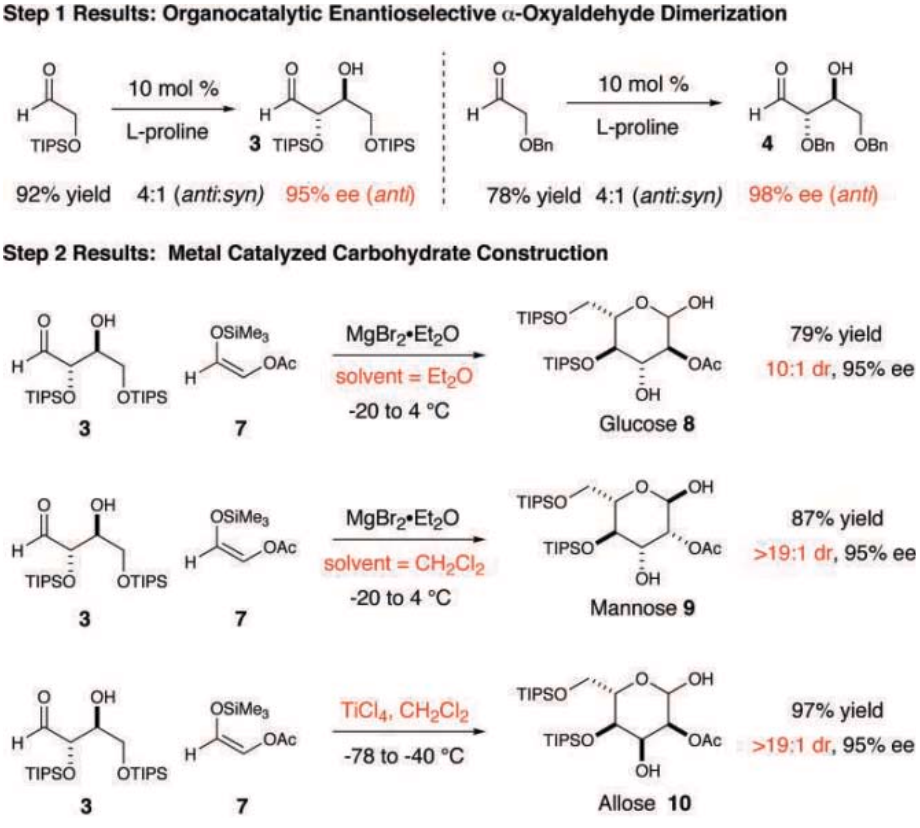


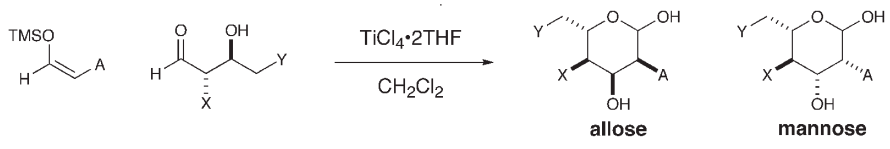
Fig. 2. Step 1 Results: The enantioselective dimerization of α-oxyaldehydes. Step 2 Results: The Lewis acid–mediated Mukaiyama aldol-carbohydrate cyclization.

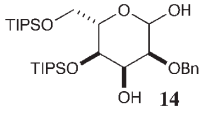
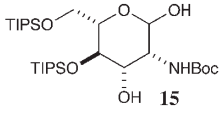
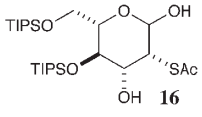
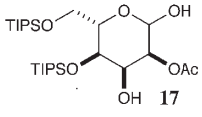
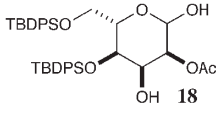
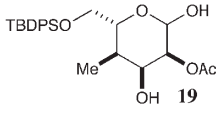
enolsilane **7** in the presence of Lewis acidic salts such as $MgBr_2{\cdot}OEt_2$ or $TiCl_4$ (Fig. 2, Step 2 results). Preliminary studies revealed that this second aldol reaction does provide polyol-differentiated hexose carbohydrates in excellent yields and diastereoselectivities (dr) (*18*). More important, selective access to either glucose, mannose, or allose can be accomplished by judicious choice of Lewis acid and reaction solvent.

For example, the use of $MgBr_2{\cdot}OEt_2$ in solvents such as ether, toluene, or pentane affords high levels of selectivity for glucose **8** (8:1 to 10:1), whereas the analogous reaction in dichloromethane is selective for mannopyranose **9**. Using optimized conditions, we obtained a 79% yield and a 10:1 preference for glucose in diethyl ether, whereas we observed an 87% yield and >19:1 selectivity for mannose in dichloromethane (Fig. 2). The origins of this dramatic change in isomer selectivity as a function of reaction solvent reflect the capacity of the reaction medium to dictate which face of the enolsilane reacts with the aldehyde. Furthermore, exposure of the same aldehyde and enolsilane components **3** and **7** to $TiCl_4$ leads to the selective formation of the allose carbohydrate isomer in >19:1 selectivity, 97% yield, and 95% ee. In this latter case, we have determined that the enolsilane undergoes transmetallation to generate a titanium-enolate before the Aldol Step 2 event. We propose that this metalloenolate participates in a cyclic (closed) transition state with the Felkin diastereoface of the aldehyde, whereas the magnesium reactions involve addition of the enolsilane to the opposite (non-Felkin) aldehyde face. We note that the unnatural (L) form of carbohydrates **8**, **9**, and **10** could be accessed selectively by using the alternate (D) enantiomer of proline in the Aldol Step 1.

Having developed this methodology, we applied our reaction sequence to the preparation of $^{13}C_6$-labeled hexoses. Specifically, we produced fully ^{13}C-labeled, differentially protected D-glucose **11**, D-mannose **12**, and D-allose **13** derivatives in only four linear steps from $^{13}C_2$-ethylene glycol (*19*), in overall yields of 33%, 35%, and 43%, respectively.

Table 1. Representative two-step enantioselective carbohydrate synthesis. Temperature refers to the final temperature of the reaction mixture after being warmed from –78°C. Yield refers to the combined yield of diastereomers. Diastereoselectivity (dr) was determined by proton nuclear magnetic resonance (^1H NMR) integration of the reaction mixture. Entry 4 was performed with $TiCl_4$.



Entry	A	X	Y	Major isomer	Temp (°C)	% yield	dr	%ee
1	OBn	OTIPS	OTIPS	 **14**	–30	83	>19:1	95
2		OTIPS	OTIPS	 **15**	–40	74	10:1 (mannose)	95
3	SAc	OTIPS	OTIPS	 **16**	–20	71	19:1 (mannose)	95
4	OAc	OTIPS	OTIPS	 **17**	–40	96	>19:1	95
5	OAc	OTBDPS	OTBDPS	 **18**	–20	86	>19:1	96
6	OAc	Me	OTBDPS	 **19**	–30	68	>19:1	99

Our route to differentiated hexoses is also amenable to considerable structural variation in both the enolsilane reagent and the β-oxyaldehyde component (Table 1). This critical feature in reaction versatility allows the rapid construction of hexoses that can be directly used in the synthesis of di- or polysaccharides. For example, carbohydrates that contain participating or nonparticipating groups at the C(2) position are readily accessed by using the respective acyloxy- or benzyloxy-substituted enolsilanes (Table 1, entry 1, A = OBn, 83% yield, >19:1 allose selective; entry 4, A = OAc, 96% yield, >19:1 allose selective). Such hexose systems have established utility as either α- or β-coupling partners in polysaccharide synthesis (*1*, *2*). The modular nature of the Aldol Step 1 also allows for broad diversification of substituents at the carbohydrate C(4) and C(6) positions (*10*, *16*). For example, the incorporation of TIPS-protecting and tertiary-butyldiphenylsilyl (TBDPS)–protecting groups at these sites is readily accomplished (Table 1, entries 4 and 5, 86 to 96% yield, >19:1 dr, ≥95% ee). These protecting groups can be selectively removed from the C(6) position, thereby affording carbohydrates that are differentially protected at each hydroxyl site. As such, these versatile saccharide monomers can be rapidly manipulated to expose the C(2), C(3), C(4), or C(6) hydroxyl groups, an important consideration for di- or polysaccharide couplings.

The reaction sequence also allows rapid access to a wide variety of unnatural carbohydrates that substitute carbon, nitrogen, and sulfur groups for the native hydroxy constituents. The analogous reactions using amino- and thio-substituted enolsilanes provide the mannose architecture in high selectivity, affording the 2-*tert*-butylcarba-moylmannose derivative **15** (Table 1, entry 2) in 74% yield and 10:1 diastereocontrol and the 2-acetylmercaptomannose product **16** (Table 1, entry 3) in 71% yield and >19:1 mannose selectivity. Carbogenic substituents can also be introduced at the saccharide C(4) position in the case where α-alkyl and α-oxy aldehydes were cross-coupled in the Step 1 Aldol event (Table 1, entry 6, 68% yield, >19:1 dr, 99% ee). The capacity to selectively build known carbohydrates with single-point atomic mutations will enable medicinal chemists to rapidly study structure activity relationships (SAR) on mono-, di-, and polysaccharide templates.

Our strategy for the synthesis of differentially protected hexoses thus provides rapid enantioselective access to key building blocks in saccharide and polysaccharide synthesis. Furthermore, our approach efficientlyyields isotopic and functional variants of the hexoses that have not been readily accessible for pharmaceutical study.

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21. Financial support was provided by the NIH National Institute of General Medical Sciences (R01 GM66142-01) and gifts from Bristol-Myers Squibb, Johnson and Johnson, Eli Lilly, and Merck Research Laboratories. D.W.C.M. is grateful for support from the Camille and Henry Dreyfuss Foundation, the Sloan Foundation, and Research Corporation under the Cottrell Scholarship and Research Innovation programs. A.B.N. is grateful for an NSF predoctoral fellowship.

Supporting Online Material
www.sciencemag.org/cgi/content/full/1101710/DC1
Materials and Methods

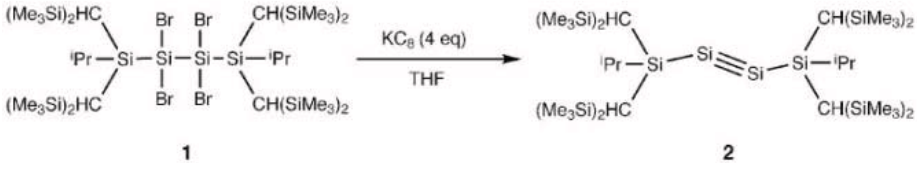
21 June 2004; accepted 22 July 2004
Published online 12 August 2004;
10.1126/science.1101710
Include this information when citing this paper.

A Stable Compound Containing a Silicon-Silicon Triple Bond

Akira Sekiguchi,* Rei Kinjo, Masaaki Ichinohe

The reaction of 2,2,3,3-tetrabromo-1,1,4,4-tetrakis[bis(trimethylsilyl)methyl]-1,4-diisopropyltetrasilane with four equivalents of potassium graphite (KC_8) in tetrahydrofuran produces 1,1,4,4-tetrakis[bis(trimethylsilyl)methyl]-1,4-diisopropyl-2-tetrasilyne, a stable compound with a silicon-silicon triple bond, which can be isolated as emerald green crystals stable up to 100°C in the absence of air. The Si≡Si triple-bond length (and its estimated standard deviation) is 2.0622(9) angstroms, which shows half the magnitude of the bond shortening of alkynes compared with that of alkenes. Unlike alkynes, the substituents at the Si≡Si group are not arranged in a linear fashion, but are trans-bent with a bond angle of 137.44(4)°.

Hydrocarbons containing C=C double bonds (alkenes) and C≡C triple bonds (alkynes) form an abundant and structurally diverse class of organic compounds. However, the ability of heavier congeners of carbon (where element E is Si, Ge, Sn, and Pb) to form double bond of the type >E=E< and triple bond of the type -E≡E- was for a long time doubted (*1–4*). The first attempts to generate such species were unsuccessful, resulting in the formation of polymeric substances. This led to the oftencited "double-bond rule": Those elements with a principal quantum number equal to or greater than three are not capable of forming multiple bonds because of the considerable Pauli repulsion between the electrons of the inner shells (*5–7*). Such a viewpoint prevailed despite the accumulation of a vast amount of experimental data supporting the existence of multiply bonded species as reactive intermediates (*1–4*). This conflict was resolved nearly 30 years ago, when Lappert and Davidson reported the synthesis of the stable distannene $[(Me_3Si)_2CH]_2Sn{=}Sn[CH(SiMe_3)_2]_2$, where Me is methyl, which has a Sn=Sn double bond in the solid state (*8*). The next important discoveries came from two research groups in 1981: West and colleagues reported the synthesis of a stable compound with a Si=Si double bond, tetramesityldisilene (*9*), and Brook *et al*. synthesized a compound with a Si=C double bond (*10*). As for triple bonds, Power and co-workers recently prepared alkyne analogs of the heavier group 14 elements: germanium, tin, and lead (*11–13*). However, despite bearing nominal triple bonds, these compounds actually exhibited a highly pronounced nonbonding electron density character at the central atoms, resulting in a decrease in the bond order on descending group 14 (*14*, *15*). In light of these results, isolation of the silicon analog of alkynes has been a compelling goal. Although the theoretical analysis predicted the experimental accessibility of disilynes with a silicon-silicon triple



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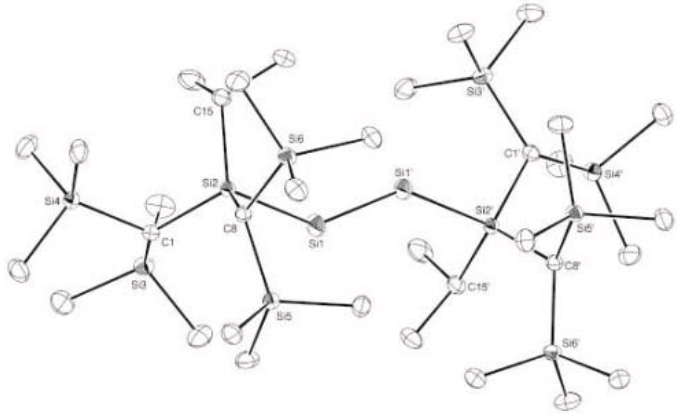

Fig. 1. Molecular structure of 1,1,4,4-tetrakis[bis(trimethylsilyl)methyl]-1, 4-diisopropyl-2-tetrasilyne (**2**) (30% probability ellipsoids for Si and C). Selected bond lengths (Å): Si1–Si1′ = 2.0622(9), Si1–Si2 = 2.3698(6), Si2–C1 = 1.9119(15), Si2–C8 = 1.9120(15), and Si2–C15 = 1.9180(16). Selected bond angles (°): Si1′–Si1–Si2 = 137.44(4), Si1–Si2–C1 = 108.97(5), Si1–Si2–C8 = 108.38(5), Si1–Si2–C15 = 106.47(5), C1–Si2–C8 = 106.83(6), C8–Si2–C15 = 114.77(7), and C1–Si2–C15 = 111.30(7). Estimated standard deviations are in parentheses.

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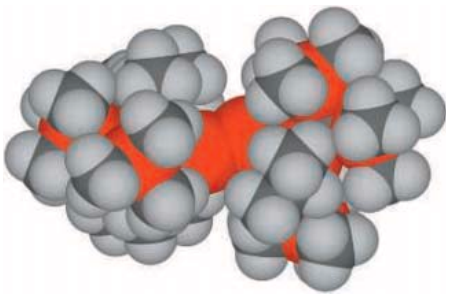

Fig. 2. Space-filling model of **2.** Si, red; C, gray; H, white.

bond (*16*), all attempts to isolate such postulated molecules have been unsuccessful (*17*–*19*). The difficulty in synthesizing disilynes is due in large part to their high reactivities, especially toward isomerization and dimerization.

We report the isolation and full characterization of 1,1,4,4-tetrakis[bis(trimethylsilyl)methyl]-1,4-diisopropyl-2-tetrasilyne **2**, a stable disilyne R-Si≡Si-R, which is the Si analog of an alkyne. In this compound, the Si≡Si triple bond is kinetically and thermodynamically stabilized by two large silyl substituents, each bearing one isopropyl and two bis(trimethylsilyl)methyl groups. The compound is accessed by reduction of a tetrabrominated precursor. Thus, the reaction of 2,2,3,3-tetrabromo-1,1,4,4-tetrakis[bis(trimethylsilyl)methyl]-1,4-diisopropyltetrasilane **1** with four equivalents of potassium graphite (KC_8) in dry tetrahydrofuran (THF) produces a dark green mixture, from which disilyne **2** can be isolated as extremely air- and moisture-sensitive emerald green crystals in 73% isolated yield (Reaction 1) (*20*, *21*). Despite the large steric congestion, the debromination reaction proceeds rapidly and cleanly. The disilyne **2** was purified by recrystallization from pentane at –30°C; it has a decomposition point of 127° to 128°C. No evidence for the isomerization of **2** to RRSi=Si or dissociation into the two RSi fragments (where R is $Si^iPr[(CH(SiMe_3)]_2$ and ^{i}Pr is isopropyl) was observed, indicating that the two central Si atoms are strongly bonded.

The disilyne **2** was fully characterized spectroscopically; the most informative data came from ^{29}Si nuclear magnetic resonance (NMR) studies. Four equal-intensity resonance signals at the chemical shifts δ = –0.3, 0.0, 20.7, and 89.9 parts per million (ppm) were observed in the ^{29}Si NMR spectrum, assigned as follows: The peak at 89.9 ppm corresponds to a triply bonded Si atom, the peak at 20.7 ppm corresponds to Si atoms bonded to the Si≡Si group, and peaks at –0.3 and 0.0 ppm correspond to the four $CH(SiMe_3)_2$ groups (*21*). The resonance of the sp-hybridized Si atoms is shifted upfield compared with that of silyl-substituted disilenes (δ = 142.1 to 154.5 ppm) (*22*), as was observed in the case of ^{13}C NMR chemical shifts of silyl-substituted alkenes (δ = 188 to 197 ppm) and alkynes (δ = 112 to 114 ppm) (*23*).

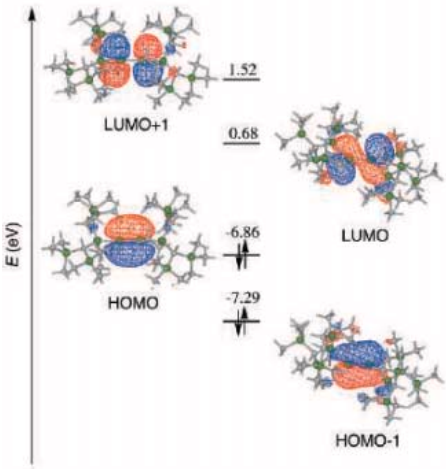

Fig. 3. The π MOs of **2** calculated at the HF/6-311G(d)//B3LYP/6-31G(d) level (HOMO-1, HOMO, LUMO, and LUMO+1). E is the energy level of the orbitals. The large vertical arrow indicates the energy level. The two small arrows indicate two electrons of opposing spin, referred to as paired electrons.

The solid state structure of **2** was confirmed by x-ray crystallographic analysis (Fig. 1). Full metrical parameters are listed in table S3 (*24*). The four Si atoms (Si2, Si1, Si1′, and Si2′) are perfectly coplanar and the bulky $Si^iPr[(CH(SiMe_3)]_2$ groups protect the central Si≡Si triple bond. The most significant result is the Si≡Si triple-bond length of 2.0622(9) Å. This value is 3.8% shorter than typical Si=Si double-bond length (2.14 Å) and 13.5% shorter than the average Si-Si single-bond length of 2.34 Å (*4*). This shortening is half the magnitude of that in the carbon counterparts. Moreover, alkynes have a linear geometry around the C≡C triple bond, whereas disilynes have been predicted to have a highly pronounced trans-bent geometry around the Si≡Si triple bond (*14*, *16*). The structure confirms this prediction: The substituents at the Si≡Si are not arranged in a linear fashion, but are trans-bent with a bond angle of 137.44(4)°, as determined by the Si2-Si1-Si1′ angle. This bond angle is 12.5° smaller than that calculated for HSi≡SiH (124.9°). According to theoretical investigations, substitution by electropositive silyl groups leads to a less trans-bent disilyne structure (*25*). The structure of **2** presented here is close to that predicted by a recent density functional (DFT) calculation on $(^tBu_3Si)_2MeSiSi{\equiv}SiSiMe(Si^tBu_3)_2$, where ^{t}Bu is *tert*-butyl (*26*).

The space-filling model of **2** shown in Fig. 2 highlights the steric protection of the Si≡Si group by the isopropyl and bis(trimethylsilyl)methyl substituents. Upon replacement of the isopropyl groups in precursor with methyls, the reaction to produce the disilyne yields a dimerization product instead, bearing a tetrasilatetrahedrane core (*27*).

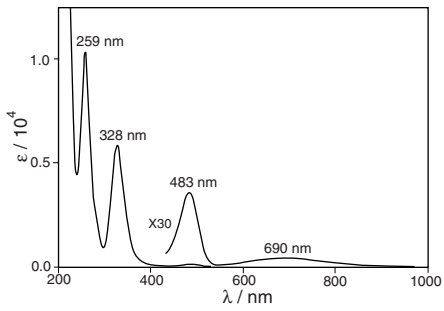

Fig. 4. Ultraviolet-visible absorption spectrum of **2** in hexane at room temperature; ε is the molar extinction coefficient ($dm^3\ mol^{-1}\ cm^{-1}$).

Despite the steric protection, the Si≡Si triple bond in **2** does undergo addition reactions with a halogen. Thus, **2** readily reacted with two equivalents of bromine at room temperature in hexane to form **1** in 94% yield by cleavage of the two π(Si≡Si) bonds.

A DFT calculation on disilyne **2** at the B3LYP/6-31G(d) level of theory well reproduces the experimental geometry and the structural parameters (calculated value: 2.093 Å for the Si≡Si bond length, 136.1° for the trans-bending angle). The molecular orbitals (MOs) of **2** calculated at the HF/6-311G(d)//B3LYP/6-31G(d) level presented in Fig. 3 show two nondegenerate highest occupied π MOs (HOMO-1 and HOMO) and two lowest unoccupied antibonding π* MOs (LUMO and LUMO+1). The out-of-plane HOMO and LUMO+1 are represented by the pure (p_z-p_z) π MOs, whereas the in-plane HOMO-1 and LUMO are represented mainly by (p_y-p_y) π MOs with a slight contribution from the antibonding σ* (Si-Si) orbital of the central bond. In accordance with the triple-bond structure, natural bond orbital analysis of **2** shows electron occupation of the two π(Si≡Si) orbitals (1.934 and 1.897 electron), indicating their bonding character. The bond order (Wiberg bond index) of Si1-Si1′ is 2.618, which agrees with the real Si≡Si triple bond. The presence of the two nondegenerate π and two π* MOs in **2** is reflected in the ultraviolet-visible absorption of **2**, as shown in Fig. 4. The strong absorption bands at wavelengths (λ) of 259 and 328 nm are due to the two allowed π-π* transitions. The weak absorption bands with maxima of 483 and 690 nm are probably a result of forbidden transitions, and the latter very weak one (HOMO-LUMO transition) is responsible for the emerald green color of **2**.

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19. The reduction of (^{t}Bu$_3$Si)$_2$MeSiClSi=SiClSiMe(Si^{t}Bu$_3$)$_2$ with lithium naphthalene in THF has recently been reported (*28*). This contains an unstable product with a low field ^{29}Si NMR signal at δ = 91.5 ppm, which was ascribed to a Si≡Si triple-bond resonance.
20. Crystals of **1** (100 mg, 0.087 mmol) and KC_8 (50 mg, 0.370 mmol) were placed in a glass tube and degassed. Dry oxygen-free THF (2 ml) was introduced by vacuum transfer and the mixture was allowed to warm from –78°C to room temperature overnight with stirring. The solution turned an intense green color. The solvent was replaced by hexane, and then the resulting potassium salt and graphite were filtered off in a glove box (a box equipped with gloves in which air- and moisture-sensitive compounds can be handled). After evaporation of the solvent, pentane was added and the solution was cooled at –30°C to give emerald green crystals of **2** (53 mg, 73%).
21. NMR of **2** ([D_6]benzene solution, ^{1}H) δ values (ppm): –0.01 (singlet, 4H), CH protons; 0.39 (singlet, 36H), $SiMe_3$ protons; 0.57 (singlet, 36H), $SiMe_3$ protons, 1.44 (doublet, 12H, spin-spin coupling constant J = 6.0 Hz, 12H), isopropyl methyl protons, 1.49 (septet, spin-spin coupling constant J = 6.0 Hz, 2H), isopropyl methyne proton. NMR (^{13}C) δ values: 5.1, 5.7, 8.9, 17.8, and 22.3. NMR (^{29}Si) δ values: –0.3, 0.0, 20.7, and 89.9. High-resolution mass spectrum: mass-to-charge ratio (*m/z*) calculated for $C_{34}H_{90}Si_{12}$ to be 834.4274, experimentally found to be 834.4275. Ultraviolet-visible spectrum (in hexane solution): λ_{max} [wavelength, molar extinction coefficient (ε)/dm^3 mol^{-1} cm^{-1}] 259, 10300; 328, 5800; 483, 120; and 690, 14.
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24. An emerald green crystal (approximate dimensions, 0.30 by 0.15 by 0.15 mm) of **2** was used for the x-ray diffraction data collection on a Mac Science DIP2030K Image Plate Diffractometer with graphite-monochromatized Mo–K_α radiation (λ = 0.71070 Å). Cell constants and an orientation matrix for data collection corresponded to the monoclinic space group *C2/c*, with *a* = 30.9620(11) Å, *b* = 10.9060(2) Å, *c* = 18.1170(7) Å, β = 118.995(2)°, *V* = 5350.8(3) Å^3, four molecules per unit cell, formular weight 836.14, and calculated density 1.038 Mg/m^3. Data were collected at 120 K, θ range from 2.18° to 28.01°. There were 26,993 collected reflections (6412 unique, R_{int} = 0.0290); R_1 = 0.0373 for 5479 reflections with $I > 2\sigma(I)$, wR_2 = 0.1096 for all reflections. More crystallographic data are available at the Cambridge Crystallographic database, accession code and deposition no. CCDC 245523.
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29. Supported by a Grant-in-Aid for Scientific Research (Nos. 134078204, 16205008, and 16550028) from the Ministry of Education, Science and Culture of Japan, and Center of Excellence program. We thank S. Nagase for helpful discussions and advice.

Supporting Online Material
www.sciencemag.org/cgi/content/full/305/5691/1755/DC1
Tables S1 to S5

30 June 2004; accepted 2 August 2004

A Linear, O-Coordinated η^1-CO_2 Bound to Uranium

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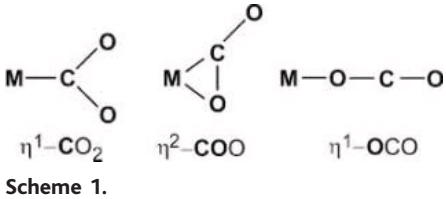
The electron-rich, six-coordinate tris-aryloxide uranium(III) complex [((^{Ad}ArO)$_3$tacn)U^{III}] [where (^{Ad}ArOH)$_3$tacn = 1,4,7-tris(3-adamantyl-5-*tert*-butyl-2-hydroxybenzyl)1,4,7-triazacyclononane] reacts rapidly with CO_2 to yield [((^{Ad}ArO)$_3$tacn)U^{IV}(CO_2)], a complex in which the CO_2 ligand is linearly coordinated to the metal through its oxygen atom (η^1-O**CO). The latter complex has been crystallographically and spectroscopically characterized. The inequivalent O–C–O bond lengths [1.122 angstroms (Å) for the O–C bond adjacent to uranium and 1.277 Å for the other], considered together with magnetization data and electronic and vibrational spectra, support the following bonding model: U^{IV}=O=C$^{\bullet}$–O$^-$ ↔ U^{IV}–O≡C–O$^-$. In these charge-separated resonance structures, the uranium center is oxidized to uranium(IV) and the CO_2 ligand reduced by one electron.**

Carbon dioxide has been implicated as a main contributor to global warming because of its role in radiative forcing (*1*). However, CO_2 also represents an abundant renewable resource for the production of fine chemicals and clean fuels. Interest in metal-mediated multielectron reduction of CO_2 therefore remains high, but the molecule's inherent thermodynamic stability hinders the development of metal catalysts that achieve CO_2 activation and functionalization.

Particularly intriguing for synthetic chemists is the discovery of relatively simple coordination complexes that bind CO_2 and facilitate its reduction (*2*). Chemists have isolated and structurally characterized several synthetic metal complexes of CO_2, such as Aresta's archetypal [(Cy_3P)$_2$Ni(CO_2)] (Cy = cyclohexyl) (*3*, *4*) and Herskowitz's [(diars)$_2$M(CO_2)(Cl)] [diars = *o*-phenylenebis(dimethylarsine); M = Ir, Rh] (*5*), featuring the bidentate η^2-**CO**O and carbon-bound η^1-**C**O_2 binding modes, respectively. Activation of CO_2 via its adsorption on metal surfaces is of considerable interest for catalysis at the gas-surface interface (*6*, *7*). Most recently, Andrews and co-workers studied the interaction of CO_2 with a variety of transition (*8*) and actinide (*9*) metal atoms generated via laser ablation. Although C–O bond cleavage of a proposed intermediate η^2-**CO**O complex is predominant in these surface-adsorbed systems, there also is spectral evidence for η^1-**O**CO adsorption in low-temperature matrices (Scheme 1) (*8*). The most important CO_2 activation process occurs naturally during photosynthesis. It was proposed that during photosynthetic CO_2 fixation, an oxygen-coordinated CO_2 ligand (η^1-**O**CO) is enzymatically reduced by ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) (*10*). Oxygen coordination appears to be an indispensable step for C-functionalization in this system. Recently, the relevance of the η^1-**O**CO coordination mode for biological systems was fueled by a crystallographic study on a deacetoxycephalosporin C synthase (DAOCS) mutant. The presence of electron density in proximity to the active site's iron center was found to be consistent with a monodentate O-bound CO_2 molecule (*11*). However, definitive structural characterization of inorganic coordination complexes with a linear oxygen-bound η^1-**O**CO coordination mode has remained elusive (*2*).



Scheme 1.

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The large negative reduction potential and oxophilicity of trivalent uranium complexes can be applied to CO_2 fixation. Coordination complexes of uranium have undergone a renaissance in the past few years (*12*). It has been shown that, if stabilized by a suitable supporting ligand set, uranium compounds are highly reactive (*13*) and can engage in the formation of carbon monoxide (*14*, *15*), dinitrogen (*16*, *17*), and even alkane complexes through *f*-orbital interaction (*18*).

Here, we report carbon dioxide coordination by a uranium compound resulting in reductive activation of the CO_2 ligand. For the exploration of CO_2 coordination and redox chemistry with a reactive uranium center, we sought to use the electron-rich uranium(III) complex [((^{Ad}ArO)$_3$tacn)U^{III}] [**1**, (^{Ad}ArOH)$_3$tacn = 1,4,7-tris(3-adamantyl-5-*tert*-butyl-2-hydroxybenzyl)1,4,7-triazacyclononane] (*19*).

The uranium center of coordinatively unsaturated **1** is located deep inside a cavity formed by the hexadentate aryloxide-functionalized polyamine chelator. Intramolecular hydrophobic interactions between the hydrocarbyl substituents displace the U ion below the trigonal plane of the three aryloxide ligands. The sterically demanding adamantyl groups form a narrow cylindrical cavity above the uranium ion, thereby providing restricted access to an incoming ligand and protecting the uranium center from bimolecular decomposition reactions.

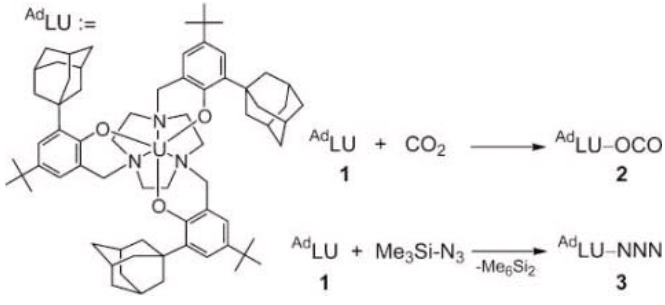
Exposure of toluene solutions or even solid samples of intensely colored **1** to CO_2 gas (1 atm) results in instantaneous discoloration of the samples (*20*). After filtration and concentration of the toluene reaction mixture, colorless crystals of [((^{Ad}ArO)$_3$tacn)U^{IV}(CO$_2$)] (**2**) are isolated in ~50% yield (Scheme 2). The infrared spectrum of the crystalline sample in Nujol clearly exhibits a distinct vibrational band centered at 2188 cm^{-1}, indicative of a coordinated and significantly activated CO_2 ligand. When **1** is exposed to isotopically labeled $^{13}CO_2$ gas, this band shifts to 2128 cm^{-1} (fig. S1). The $^{12}C/^{13}C$ isotopic ratio *R* (2188/2128) of 1.0282 is close to that of free CO_2 gas (*R* = 1.0284), indicative of a molecule that has the linear geometry of free CO_2 as well as the same carbon motion, $\nu_3(\nu_{as}OCO)$, in this vibrational mode.

An x-ray diffraction analysis of single crystals obtained from a mixture of methylene chloride and diethyl ether confirms the presence of a coordinated carbon dioxide ligand (*21*). Unexpectedly, the CO_2 ligand in [((^{Ad}ArO)$_3$tacn)U^{IV}(CO$_2$)] · 2.5 Et$_2$O (**2** · 2.5 Et$_2$O) is coordinated to the uranium ion in a linear, oxygen-bound η^1-**O**CO fashion (Fig. 1). This CO_2 coordination mode likely is enforced by the adamantyl substituents of the supporting ligand platform. The U−OCO group has a U−O bond length of 2.351(3) Å (here and below, values in parentheses are errors in the last significant digit); the neighboring C–O bond length is 1.122(4) Å; and the terminal C−O bond length is 1.277(4) Å. The U−O−C and O−C−O angles of 171.1(2)° and 178.0(3)°, respectively, are close to linear. These metric parameters, together with the frequency redshift of the vibrational bands (ν_3:$\nu_O{}^{12}{}_{CO}$ = 2188 cm^{-1}, $\nu_O{}^{13}{}_{CO}$ = 2128 cm^{-1}), strongly suggest a molecular structure with charge-separated resonance structures U^{IV}=O=C$^{\bullet}$–O^{-} ↔ U^{IV}–O≡C–O^{-}. Such an electronic structure would result from a metal-centered one-electron oxidation upon CO_2 coordination.

This bonding assignment is further supported by comparison of complex **2** with a structurally similar azido complex (Fig. 2). Uranium(III) complex **1** reacts with one-electron oxidizers, such as trimethylsilyl azide (*22*), benzyl chloride, benzyl bromide, and iodine to form the respective seven-coordinate halide and pseudohalide U^{IV} complexes [((^{Ad}ArO)$_3$tacn)U^{IV}(X)] [X = N$_3$ (**3**), Cl, Br, I] (*20*). The azido ligand, which typically adopts a bent orientation at metal centers, is linearly coordinated in complex **3** [∠(U−N$^{\alpha}$−N$^{\beta}$) = 175.6(3)°, ∠(N$^{\alpha}$−N$^{\beta}$−N$^{\gamma}$) = 177.2(5)°, *d* (U−N$^{\alpha}$) = 2.372(3) Å, *d* (N$^{\alpha}$−N$^{\beta}$) = 1.128(4) Å, *d* (N$^{\beta}$−N$^{\gamma}$) = 1.147(5) Å] (*21*). As with the CO_2 coordination mode in **2**, this bonding motif is most likely imposed by the sterically encumbering substituents. The azido ligand allows estimation of the cavity depth of the supporting ligand framework, which is approximately equal to the sum of the *d* (U−N) and *d* (N−N) bond distances (4.65 Å).

Comparison of the CO_2 and azido complexes **2** and **3** with the starting compound **1** reveals that after axial ligand coordination, the central uranium ion is situated closer to the trigonal planar aryloxide ligand environment. Whereas the uranium(III) ion in **1** is located 0.88 Å below the ligand plane, the uranium center in **2** and **3** is displaced only 0.274 Å and 0.291 Å below the plane, respectively. The average U−N$_{tacn}$ and U−O$_{ArO}$ distances in **3** were determined to be 2.659(3) and 2.155(2) Å and thus are almost identical to those found in **2** [2.673(2) and 2.157(2) Å]. The U−O$_{ArO}$ bond distances in **2** and **3** are significantly shorter than those found in the parent trivalent complex **1** [2.226(9) Å], as well as U^{III} complexes of the related ($^{t\text{-}Bu}$ArO)tacn ligand system (*23*), namely [(($^{t\text{-}Bu}$ArO)$_3$tacn)U^{III}(CH$_3$CN)] [2.265(5) Å] (*22*) and [(($^{t\text{-}Bu}$ArO)$_3$tacn)U^{III}(^{Me}Cy-C6)] [2.244(3) Å] (*18*). Given the assignment of the valence in **3** as U^{IV} with an N$_3^-$ azido ligand and the structural similarities of complexes **2** and **3**, the oxidation state for the uranium center in **2** is also assigned as U^{IV} with a CO_2^- ligand.

The electronic structure of **2** was examined by comparison of superconducting quantum in-



Scheme 2. Synthesis of complexes.

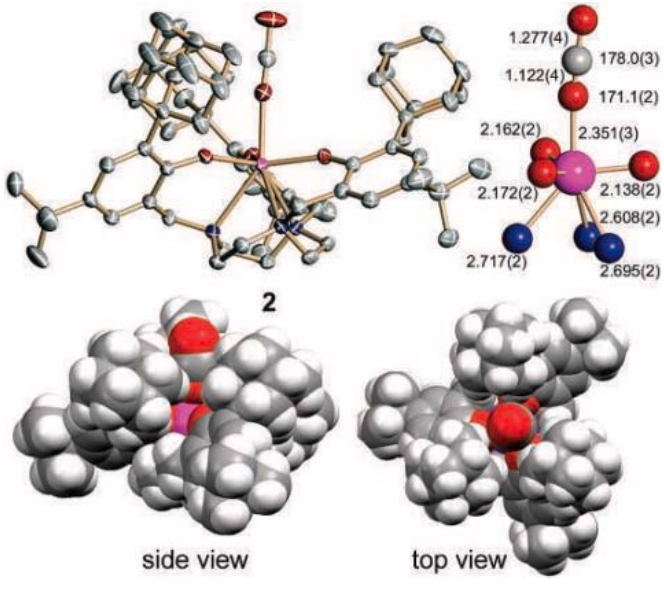


Fig. 1. ORTEP (Oak Ridge thermal ellipsoid plot) of uranium CO_2 complex **2** and in crystals of **2** · 2.5 Et$_2$O (top left) with core structure and metric parameters in angstroms and degrees (top right) and space-filling representations (bottom). Uranium, magenta; nitrogen, blue; oxygen, red; carbon, gray.

terference device (SQUID) magnetization data and electronic absorption spectra of **1**, **2**, and **3** (*20*). Over the temperature range 5 to 300 K, solid samples of **2** display a distinctly temperature-dependent magnetic moment (Fig. 3).

The magnetic moment μ_{eff} of **2** was determined to be 2.89 μ_B (where μ_B is in units of Bohr magnetons) at 300 K; it slowly decreases with decreasing temperatures, reaching a value of 2.6 μ_B at 100 K. Below 100 K, μ_{eff} decreases rapidly, reaching a value of 1.51 μ_B at 5 K. Between 300 K and 75 K, the temperature dependence of the μ_{eff} value of **2** shows a curvature reminiscent of data obtained for the U^{IV} complex **3**. Although the room-temperature μ_{eff} value of **2** is close to that found for the azide complex **3**, the low-temperature value is similar to that of the U^{III} (f^3) starting material **1** (1.73 μ_B at 5 K), which has a doublet ground state at low temperatures (*19*). Generally, U^{IV} complexes with an f^2 ($^3H_4$) electron configuration have a singlet ground state that exhibits temperature-independent paramagnetism at very low temperatures, resulting in μ_{eff} values of ~0.5 μ_B like that of **3** (fig. S2) (*20*). The CO_2 complex **2**, however, has a significantly greater μ_{eff} value at low temperature, which reinforces the description of the CO_2 ligand as one-electron reduced $CO_2^{\bullet-}$ radical anion coordinated to a U^{IV} ion. The transferred charge (virtually one unpaired electron) is localized on the coordinating CO_2 ligand. In contrast to the closed-shell N_3^- ligand of **3**, the open-shell $CO_2^{\bullet-}$ likely contributes to the observed increase in μ_{eff} value of **2** at low temperatures.

To further probe the formal oxidation state of the uranium ion, we recorded the ultraviolet/visible/near-infrared (NIR) electronic absorption spectrum of **2** and compared it to spectra of known U^{III} and U^{IV} complexes (figs. S3 and S4) (*20*). Most notably, although U^{III} and U^{V} complexes of the $(ArO)_3$tacn ligand are deeply colored (*22*), U^{IV} complexes of this ligand system, as well as the uranium-CO_2 complex **2**, appear colorless to very pale blue-green in solution and in the solid state (*22*, *23*). The absorption spectra of deep red uranium(III) complexes of the $((ArO)_3tacn)U$ system, such as **1**, are dominated by an intense absorption band centered at 455 nm (ε = 1945 M^{-1} cm^{-1}) (*20*), which can be assigned to either an allowed $d-f$ ligand field or ligand-to-metal charge-transfer transition. In addition, $f-f$ transitions give rise to a number of weak absorption bands in the NIR region between 800 and 2200 nm (ε = 50 to 150 M^{-1} cm^{-1}). In contrast, the colorless uranium(IV) complexes $[((ArO)_3tacn)U(CO_2)]$ (**2**) and $[((ArO)_3tacn)U(N_3)]$ (**3**) exhibit weak absorption bands over the entire visible and NIR region (*20*). The band intensities and positions are characteristic of the U^{IV} f^2 ion (*24*). Taken together, the metrical and spectroscopic data thus support a one-electron transfer from U to CO_2 in formation of this linearly bound, O-coordinated complex.

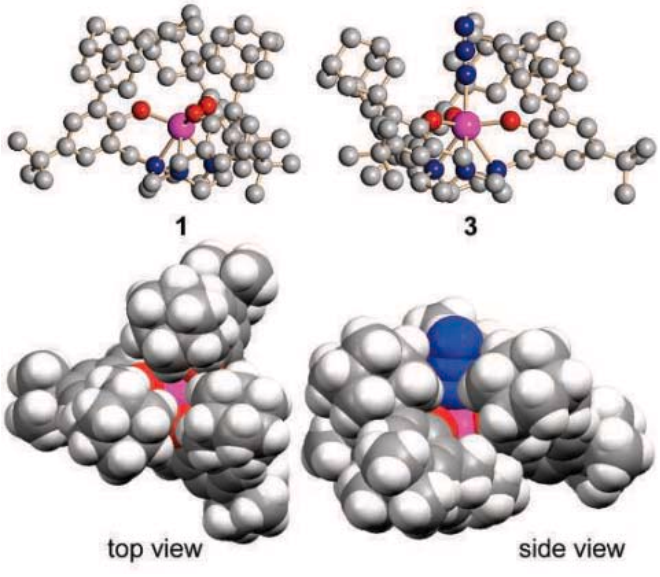


Fig. 2. Molecular structure of precursor complex **1** in crystals of **1** · C_6H_{14} (top left) and azido complex **3** in crystals of **3** · 3 CH_2Cl_2 (top right) and respective space-filling representations (bottom).

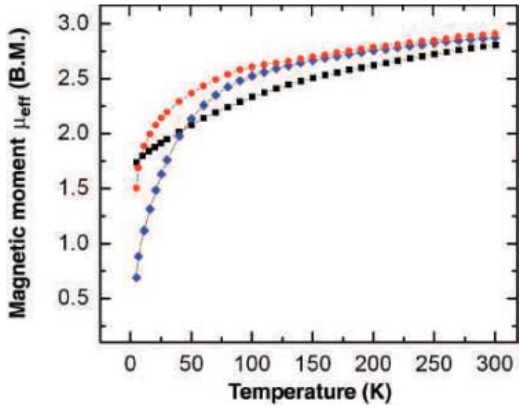


Fig. 3. Temperature-dependent SQUID magnetization data for trivalent **1** (■), uranium CO_2 complex **2** (●), and tetravalent azido complex **3** (◆) measured in the temperature range 5 to 300 K at 1 T.

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25. Supported by the University of California, San Diego, and U.S. Department of Energy grant DE-FG02-04ER15537. We thank W. L. S. Andrews and N. Edelstein for insightful discussions and NIH for a fellowship to I.C.R. (3 T32 DK07233-2651). K.M. is an Alfred P. Sloan fellow. Crystallographic data were deposited in the Cambridge Crystallographic Database Centre (**2**: CCDC-244303; **3**: CCDC-244304).

Supporting Online Material

www.sciencemag.org/cgi/content/full/305/5691/1757/DC1
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9 July 2004; accepted 13 August 2004

Age and Timing of the Permian Mass Extinctions: U/Pb Dating of Closed-System Zircons

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The age and timing of the Permian-Triassic mass extinction have been difficult to determine because zircon populations from the type sections are typically affected by pervasive lead loss and contamination by indistinguishable older xenocrysts. Zircons from nine ash beds within the Shangsi and Meishan sections (China), pretreated by annealing followed by partial attack with hydrofluoric acid, result in suites of consistent and concordant uranium/lead (U/Pb) ages, eliminating the effects of lead loss. The U/Pb age of the main pulse of the extinction is 252.6 ± 0.2 million years, synchronous with the Siberian flood volcanism, and it occurred within the quoted uncertainty.

Despite intensive study, the cause and timing of events associated with the Permian-Triassic (P-T) extinction—the most profound such extinction known over the history of life on Earth—remain uncertain. Most scenarios proposed in the past few years have invoked a catastrophic event, which almost by definition requires unusually precise geochronology for a meaningful test. Geochronology on the volcanic deposits intercalated with the sediments at the Global Stratotype Section and Point (GSSP) section in Meishan is difficult using the U/Pb method on zircon (*1*)—the most accurate available method and the only suitable mineral for U/Pb isotopic dating in these ashes. Zircon populations from these beds are typically contaminated with slightly older xenocrysts indistinguishable in appearance and corrupted by variable amounts of postdepositional Pb loss. Age lowering from the latter is pervasive (*2*) even when the surfaces of the crystals are removed by air abrasion (*2*, *3*). A previous U/Pb-zircon study (*4*), using analyses of both single- and multicrystal samples of air-abraded zircons, as well as subsequent research on extinction patterns (*5*) and using the ages in (*4*), proposed (i) an age of 251.4 ± 0.3 million years (Ma) for the main pulse of the extinction (*5*), (ii) a duration of no more than 0.165 Ma (from the sedimentation rate implied by the bracketing ages) for the carbon isotope ($\delta^{13}C$) excursion, and (iii) an age of 251 Ma for the P-T boundary (base Triassic) defined by the First Appearance Datum (FAD) of the conodont *Hindeodus parvus* (at the GSSP section in Meishan, eastern China). This data set was complex (such that most of the data were excluded from consideration). Recent time-scale compilations and most subsequent studies regarding causes and rates of the biotic crisis have adopted these ages. A subsequent study (*1*) on samples from the same ash beds but analyzing single zircons concluded that reliable U/Pb ages with such low uncertainties are difficult to obtain from such complex zircon populations, and suggested that the age of the base of the Triassic is ≥252.5 ± 0.3 Ma (on the basis of an age for an ash layer postdating the P-T boundary). The maximum age of the mass extinction was placed at 254 Ma [which was acknowledged to be "a weak conclusion" (*1*) on the basis of an incoherent data set from an ash bed at the mass extinction level]. Here we present a new analysis of single zircons from the type sections in which the pretreatment pioneered in (*6*, *7*) removes the problems of lead loss and yields coherent data sets for all of the analyzed ash layers.

Most of our samples are from the Shangsi site (32°20′N, 105°28′E, north Sichuan Province, central China). The late Permian–early Triassic sedimentary rocks are exposed in a road cut and in a parallel section in a riverbed (fig. S1C). Upper Permian limestones grade into fine-grained lowermost Triassic sandstone. As at Meishan (Zhejiang province, southeast China), volcanic ash beds are intercalated within the sediments. The succession is correlated in detail with the GSSP section in Meishan (*8*–*10*). The extinction horizon coincides with the base of bed 28 [bed numbers are according to (*8*)], just postdating ash horizon SH10(27) [bed numbers for all ash samples according to (*8*) are given in parentheses]. The first appearance of *H. parvus* is found in bed 30 at the 104.6-m level where it occurs together with *Isarcicella turgida*. The latter is regarded as the descendent of *H. parvus*, and this indicates that the true P-T boundary level (equating to the first appearance of *H. parvus* in the GSSP section at Meishan) may be some short distance below the 104.6-m level at Shangsi. The $\delta^{13}C$ record at Shangsi, although based on sparser sampling, shows a minimum of ~ −5‰, a significantly lower value than at Meishan (*5*). One study placed the $\delta^{13}C$ minimum (*11*) at Shangsi 5 m above the mass extinction horizon, whereas another (*12*) placed it with the extinction (within bed 28).

In general, the commonly used techniques for eliminating regions of zircons that have experienced Pb loss (e.g., air abrasion, leaching by hot HF at 1 atm.) are only moderately successful (*1*, *3*, *13*). However, application of a pretreatment method (*6*, *7*) involving high-temperature annealing, followed by partial HF digestion at high temperature and pressure, can be effective at removing portions of zircons that have lost Pb, without affecting the isotopic systematics of the remaining material. The aggressiveness of the technique naturally evokes concerns of laboratory artifacts in the resulting analyses. However, diffusion of Pb out of the zircons during the annealing step is ruled out by the studies of (*14*, *15*), as well as by the striking statistical coherence of the resulting U/Pb ages. Scanning electron microscopy (SEM) images (Fig. 1) of annealed and "chemically abraded" (*7*) zircon crystals show that domains affected by Pb loss are specifically removed during the partial HF-attack phase. Unlike air abrasion, this approach seems to access such domains well into the crystal interior.

We analyzed zircons from eight volcanic-ash layers of Late Permian age in the marine succession of Shangsi (north Sichuan, China) and also from the boundary clay [bed 25 in (*9*), sample D3t in this study] from the GSSP section in Meishan (Zhejiang, China). Unless otherwise stated, all ages reported here are weighted-mean $^{206}Pb/^{238}U$ ages [uncertainties are given at 2σ or 95% confidence (Fig. 2 and table S1); see (*2*) for analytical procedures].

Six zircons from sample SH01(7), from a lower Wuchiapingian ash horizon at 23.5 m, yield an age of 259.1 ± 1.0 Ma. Zircons from the lower Wuchiapingian ash layer SH03(8) (36.3 m) are small and low in U, so that, like SH01(7), uncertainties for individual analyses are relatively large. Isotopic data from all five analyzed zircon crystals define a coherent cluster, with an age of 260.8 ± 0.8 Ma. Analysis of the leachate from the combined zircons indicates that the chemically abraded portions of the crystals had lost an average of 8% of their radiogenic Pb. The weighted-mean $^{206}Pb/^{238}U$ ages of SH01(7) and SH03(8) are not in stratigraphic order. Although the error ellipses of both analyses overlap concordia, the centroid of the SH01(7) ellipse falls to the left of concordia, SH03(8) to the right. As a result, the most-probable age of SH01(7) is slightly older than

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the $^{206}Pb/^{238}U$ age, and that of SH03(8) is slightly younger. The concordia age algorithm (*16*), which makes use of all of the U/Pb isotopic ratios, errors, and error correlations at once, provides the best tool for evaluation of the SH01(7)-SH03(8) data. The concordia ages of SH01(7) and SH03(8) are 259.5 ± 0.9 Ma and 260.4 ± 0.8 Ma, respectively, indicating that an apparent reversal of age-order for the two samples is not particularly improbable [and can be used as a Bayesian constraint—see later discussion—to infer that SH01(7) is $0.06^{+0.81}_{-0.06}$ Ma older than SH03(8)].

Eight analyses of single zircons from volcanic-ash layer SH08(15) [upper Wuchiapingian (*8*)] at the 72.5-m level yield a tight cluster of analyses with an age of 257.3 ± 0.3 Ma. These zircons also are low in U, but with much larger crystal size (hence lower errors) than those from SH03(8).

Thirty-four zircons from ash layer SH16(18) (upper Wuchiapingian) were subjected to a variety of pretreatments (Fig. 1). More than half of the zircons pretreated with either air abrasion (*3*, *4*) or mild HF leaching alone (*1*) yield scattered and younger ages indicative of Pb loss. The median U concentration of untreated zircons and of those subjected to air abrasion and mild HF leaching is significantly higher than those of annealed/chemically abraded crystals, which indicates that zones high in U are preferentially removed by the latter pretreatment. All six of the analyzed leachates from individual annealed and chemically abraded zircons from SH16(18) have younger ages, implying domains with 12 to 42% Pb loss (Fig. 1).

Of the 16 annealed and chemically abraded zircons, two are slightly but resolvably older and were excluded from the mean because we assumed that they either represent xenocrysts or harbor inherited components. We so excluded only 3 of the 79 concordant analyses of this study, and none of these were from the young (Pb loss) side of the distributions. The remaining 14 analyses form a coherent cluster and yield an age of 253.7 ± 0.2 Ma.

Eight zircons from sample SH27(23) (97.1-m level), less than 3 m below the extinction horizon, yield an age of 253.2 ± 0.3 Ma. One crystal was slightly but resolvably older than the other eight and was rejected as above. Another crystal appears marginally older than the main cluster, but its inclusion with the main group does not lower the probability of fit (0.15) to an unacceptable level, and in any case has a negligible effect on the weighted-mean age and uncertainty. Seven other analyses have $^{207}Pb/^{206}Pb$ ages between 860 and 2700 Ma and thus indicate the presence of significantly older inherited zircon, almost certainly as cores.

All analyses from 12 zircons from sample SH09(25) (99.7-m level, about 0.3 m below the extinction horizon) cluster within analytical error and define an age of 252.5 ± 0.3 Ma. All five zircon analyses from ash bed SH10(27) (100.05-m level), which underlies the extinction horizon, form a coherent cluster with an age of 252.2 ± 0.4 Ma—indistinguishable from that of SH09(25).

Sample SH32(29) is from an ash layer at the 103.4-m level, 1.1 m below the first appearance of *H. parvus* but most likely within the lowermost Triassic (see above). One of the 13 analyzed zircons from SH32(29) contains an inherited component ($^{207}Pb/^{206}Pb$ age = 890 Ma). The remaining 12 yield an age of 252.5 ± 0.2 Ma. Although the crystal with the youngest age of these 12 appears just distinguishable from the other 11, the weight-

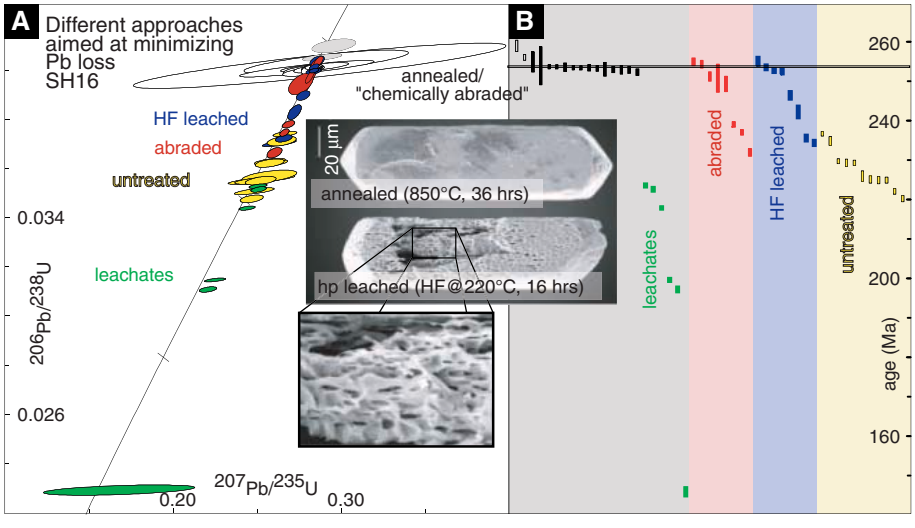

Fig. 1. Concordia diagram (**A**) and ranked ages (**B**) of zircon analyses from sample SH16(18) obtained from different approaches aimed at minimizing the effects of Pb loss. SEM image of a zircon shows the effects of chemical abrasion following annealing (uncertainties are given at the 95% confidence level).

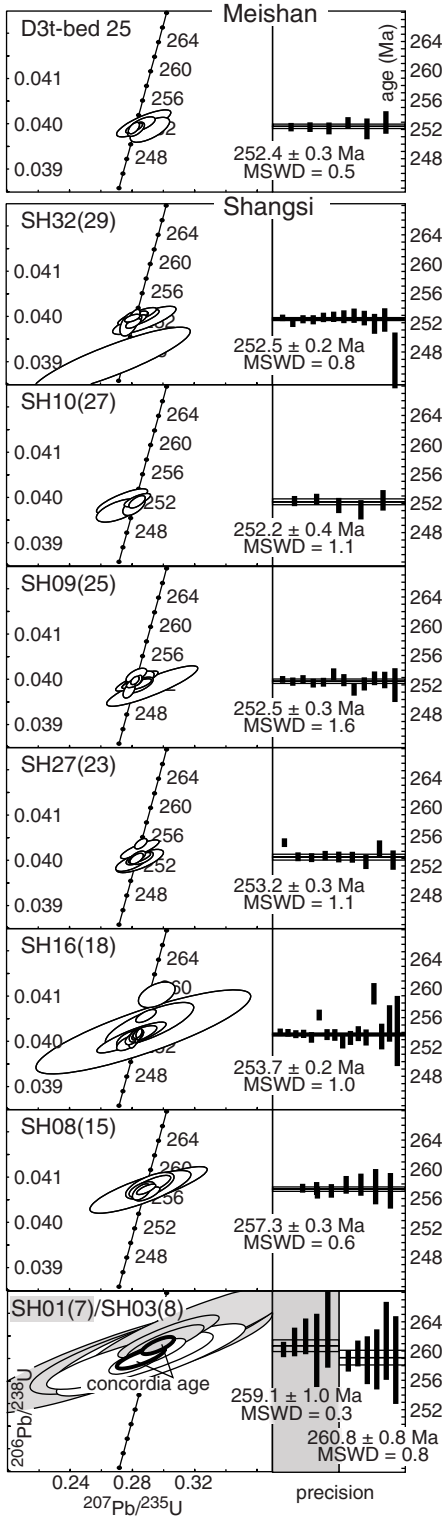

Fig. 2. Concordia diagrams (**left**) and individual $^{206}Pb/^{238}U$ ages (ranked by their uncertainties) of zircon analyses from volcanic-ash layers from Shangsi [sorted by stratigraphic age from oldest (**bottom**) to youngest (**top**)] and Meishan. SH01(7) and SH03(8) (gray) are combined, showing the overlap of the *x-y* weighted mean of the single-zircon analyses for each sample (thick outline).

ed-mean age and error are almost identical, whether or not the suspect analysis is included. The leachate of the combined sample shows that the leached parts have lost an average of 20% of their radiogenic Pb.

For comparison with the Shangsi ages, six zircons were analyzed from sample D3t (*1*) from the GSSP section in Meishan (bed 25, overlying the extinction horizon). The analyses are coherent, with an age of 252.4 ± 0.3 Ma—indistinguishable from the age of SH10(27), as well as SH09(25) and SH32(29). The stratigraphic order of the Shangsi ash horizons can be used to refine the ages and errors one more step. The methodology for doing so involves the use of Bayesian statistics and is like that applied to suites of radiocarbon ages (*17*–*19*). Prerequisites of the method for zircon geochronology are that (i) sufficient statistically coherent single-crystal replicate analyses exist to validate the assumption of a unimodal age-population, and (ii) there is little or no data culling, so that the assigned age-errors are both realistic and Gaussian.

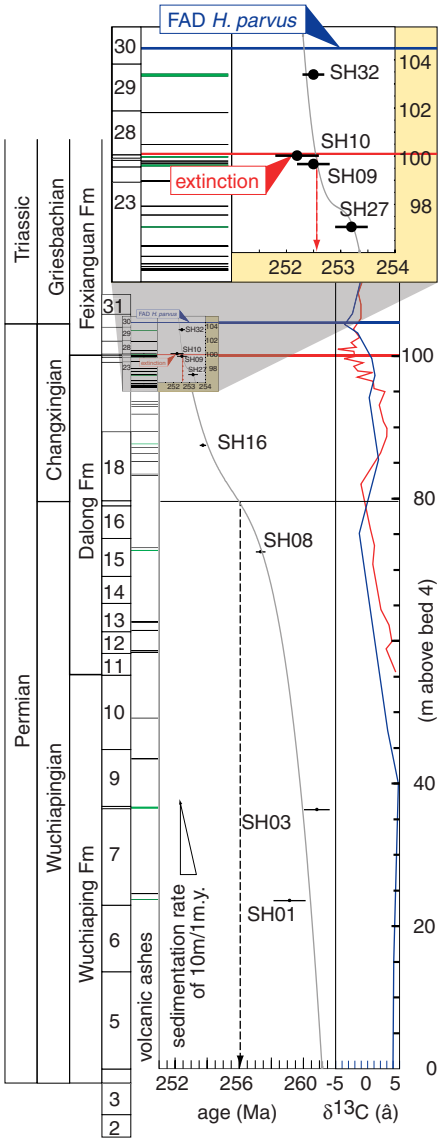

Fig. 3. Schematic diagram of the Shangsi section showing subdivision of stages, formations and bed numbers (*8*), position of ash layers, and ages versus stratigraphy including estimated sedimentation rate (third-order polynomial fit through ages is obtained from a Bayesian approach, not corrected for decompaction) as well as $\delta^{13}C$ values [(*11*), blue and (*12*), red). The P-T transition is shown in detail in the enlargement.

For our purposes, the most useful application is to determine the differences in ages between beds whose ages are otherwise unresolvable [thus circumventing the problem of the "push-apart" effect on the ages themselves (*20*, *21*)]. Using the SH27(23), SH09(25), SH10(27), and SH32(29) ages and stratigraphic order and Isoplot 3 (*22*) to perform the calculations, we find that the most-probable duration between SH09(25) and SH10(27) is less than 0.3 Ma. Similarly, the duration between SH10(27) and SH32(29) is found to be less than 0.3 Ma (that is, $0.0^{+0.3}_{-0.0}$ Ma, 95% confidence), compared to the stratigraphically unconstrained estimate of −0.2 ± 0.5 Ma. The bracketing ages of 250.7 ± 0.3 and 251.4 ± 0.3 Ma used as constraints for the age of the P-T boundary and for the duration of the negative $\delta^{13}C$ excursion in (*4*) imply a time interval of no more than 0.7 ± 0.4 Ma, but these ages are unsuitable for such a calculation, in that they are based on both multi- and single-crystal analyses and excluded data.

Because we cannot detect complexities at a level much less than the precision of the individual analyses (~3‰), we cannot resolve the presence of pervasive Pb loss at the 1‰ to 3‰ level, zircons from eruptions only a few hundred thousand years earlier, or zircons with long magma-residence times. Such considerations imply that the practical limit of the resolution of U/Pb zircon dating may not be much better—perhaps a factor of 2—than the precision of a single good analysis, with unquantified magma-residence time imposing another limit of perhaps as much as 0.3 Ma (*23*, *24*).

From these new, unambiguous ages we conclude the following: (i) The biotic crisis occurred at 252.6 Ma. The age of the biostratigraphic P-T boundary (FAD of *H. parvus* in Meishan) is slightly but unresolvably younger. This age is consistent with the previous estimate for the age of bed AW3 at Meishan [bed 28 in (*9*)], 8 cm above the P-T boundary (*1*), whereas the age of the extinction is younger than our earlier, tentative estimate (*1*). (ii) The tempo and duration of the main pulse of the P-T mass extinction are not resolvable, even with age precisions at or below the per mil level. (iii) The interpolated age of the Wuchiapingian-Changxingian boundary is 256 Ma [on the basis of third-order polynomial fit; a linear regression between SH08(15) and SH16(18) yields the same result (Fig. 3)], younger than the estimate in (*1*). (iv) The inferred age for the base of the Wuchiapingian stage and the end Guadalupian extinction is >260 Ma. (v) The carbon-isotope anomaly at Shangsi may have commenced as much as 2 million years before the P-T boundary, although the sparse available data leave this question open.

Several scenarios for the P-T extinction have been proposed and critically evaluated, including (i) a bolide impact (*25*–*29*), (ii) catastrophic environmental change caused by giant-scale volcanism (*30*, *31*), and (iii) methane release (*32*, *33*). Geochronology ($^{40}Ar/^{39}Ar$) applied to a purported impact breccia interpreted to be of P-T boundary age (*27*) shows unambiguous evidence of disturbance and, even if such evidence is ignored, is only constrained within a 20-million-year interval. The end of the Permian Period is characterized by an abundance of short and voluminous pulses of continental flood basalts, and the timing of the volcanism appears to be indistinguishable from that of the mass extinctions at the end of the Middle Permian (end Guadalupian, Emeishan basalt) and the end of the Permian [Siberian flood basalt (*30*, *31*)]. Synchrony between the end Guadalupian extinction (*34*) and the Emeishan igneous province is loosely constrained by a U/Pb sensitive high-resolution ion microprobe (SHRIMP) age of 259 ± 3 Ma for the Xinjie intrusion (*35*) and the estimate of >260 Ma for the base of the Wuchiapingian presented here, although tighter constraints are needed. The synchrony of Siberian flood volcanism with the P-T mass extinction has been demonstrated by indistinguishable $^{40}Ar/^{39}Ar$ ages of 250 Ma for the main pulse of the volcanism and for ash beds close to the extinction level from Meishan and Shangsi (*30*). $^{40}Ar/^{39}Ar$ ages based on conventional calibrations are systematically younger in the Paleozoic and Mesozoic by about 1% than U/Pb ages [most likely due to a miscalibration of the ^{40}K decay constants and data for standards (*36*)]. The $^{40}Ar/^{39}Ar$ age reported for Meishan bed 25 (*30*) recalculates to 252.1 ± 1.6 Ma based on the standard and decay-constant calibration suggested in (*37*), in agreement with the U/Pb ages presented here. We thus conclude that (i) the Siberian volcanism and the P-T extinction event are essentially synchronous (with the same scenario possibly applying to the Emeishan volcanism and the end Guadalupian extinction) and (ii) that the extinction occurred over a very short time.

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38. This study was supported by the NSF (EAR-0125799), the Australian Research Council, and the Ann and Gordon Getty Foundation. We thank J. Mattinson for generously sharing his ideas; B. Nicoll and Zhihao Wang for help and support in the field; M. Cover, A. DeHollan, and G. Johnson for lab assistance; and J. Feinberg for SEM imaging. This work is dedicated to the late Rudolf H. Steiger.

Supporting Online Material

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2 June 2004; accepted 6 August 2004

Molecular Cloud Origin for the Oxygen Isotope Heterogeneity in the Solar System

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Meteorites and their components have anomalous oxygen isotopic compositions characterized by large variations in $^{18}O/^{16}O$ and $^{17}O/^{16}O$ ratios. On the basis of recent observations of star-forming regions and models of accreting protoplanetary disks, we suggest that these variations may originate in a parent molecular cloud by ultraviolet photodissociation processes. Materials with anomalous isotopic compositions were then transported into the solar nebula by icy dust grains during the collapse of the cloud. The icy dust grains drifted toward the Sun in the disk, and their subsequent evaporation resulted in the ^{17}O- and ^{18}O-enrichment of the inner disk gas.

Oxygen is the most abundant element in the solid phases that formed early in the solar system, and it has three stable isotopes of mass numbers 16, 17, and 18. On a three-oxygen isotope diagram, $^{18}O/^{16}O$ and $^{17}O/^{16}O$ abundance ratios of most terrestrial material constitute a line with slope of ~0.5, called the terrestrial fractionation (TF) line. This slope is due to isotope fractionation processes that depend on the mass difference between each pair of isotopes. In contrast, most meteorites have oxygen isotopic compositions that diverge from the TF line (*1*). Refractory inclusions and some chondrules in primitive meteorites have the most ^{16}O-enriched isotope compositions, shifted from the TF line with magnitudes of several percent in $^{17}O/^{16}O$ and $^{18}O/^{16}O$ ratios (*1*, *2*). Nonradiogenic effects in the other major elements (e.g., Mg and Si) in these meteorite constituents have isotope compositions close to the terrestrial compositions, and their small deviations can be explained by isotope fractionation due to thermal processes, e.g., evaporation, condensation, aqueous alteration, and low-temperature chemical reaction (*3*).

The origin of mass-independent fractionation of oxygen isotopes and the lack of such fractionation in other major elements in meteorites remains poorly understood. It cannot be due to nucleosynthetic processes or nuclear reactions that involve energetic particles from the Sun or from Galactic cosmic rays, because these processes would also change the isotopic compositions of the other elements (*1*). In addition, presolar grains enriched in ^{16}O are rare in meteorites (*4*). Although some types of molecular reactions in gaseous phases have been found to induce such mass-independent isotope fractionation in oxygen (*5*), they are observed among gas species (e.g., O_3, O_2, and CO_2) that are minor in the solar nebula (*6*). Furthermore, even if such fractionation occurs, no plausible mechanism has been proposed for trapping the fractionated products into chondrite constituents. Oxygen isotope changes due to selective ultraviolet (UV) dissociation of molecules in the solar nebula gas have been proposed (*5*, *7*, *8*); however, a mechanism for transferring these effects to the chondritic constituents has not been identified.

Recently, variations in $C^{16}O/C^{18}O$ ratio have been observed in diffuse molecular clouds (*9*, *10*). These variations are explained by selective predissociation (*11*) of $C^{18}O$ by UV radiation. In the environment of molecular clouds, predissociation due to line spectrum absorption of UV photons is the dominant mechanism for photodissociation of CO (*12–17*). UV intensity at the wavelengths of dissociation lines for abundant $C^{16}O$ rapidly attenuates in the surface layer of a molecular cloud, because of its UV self-shielding. For less abundant $C^{17}O$ and $C^{18}O$, which have shifted absorption lines because of differences in vibrational-rotational energy levels, the attenuation is much slower. As a result, $C^{17}O$ and $C^{18}O$ are dissociated by UV photons even in a deep molecular cloud interior. This process results in selective enrichment of CO in ^{16}O and enrichment of atomic oxygen in ^{17}O and ^{18}O.

Because CO and atomic oxygen are the dominant oxygen-bearing gas species in molecular clouds (*18*), their isotopic fractionation may propagate to other oxygen-bearing species. Water ice is the dominant oxygen-bearing species among ices in molecular clouds (*19*), where it nucleates and grows on silicate dust grains by surface hydrogenation reactions be-

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tween atomic oxygen and hydrogen (*20*, *21*). Therefore, oxygen isotopic compositions of H_2O ice should be close to those of gaseous atomic oxygen enriched in ^{17}O and ^{18}O (*22*). Water ice is observed in molecular clouds with total visual extinction (A_V) greater than 3.2 (*23*); abundance of water ice increases with increasing A_V (*24*). As a molecular cloud becomes dense, most of the atomic oxygen reacts to form H_2O ice, and CO becomes the most dominant gas species within 10^5 years (*25*). Thus, the oxygen isotopic composition of the gas in a dense molecular cloud becomes enriched in ^{16}O with time.

Low-mass (less than two solar masses) stars form by collapse of individual cores or clumps in a cold, dark molecular cloud with molecular densities of hydrogen (n_{H2}) of 10^4 to 10^5 cm^{-3}, A_V of 5 to 25, and temperatures as low as ~10 K (*26*). According to a model simulating photochemical isotope fractionation in a molecular cloud (*17*), under these typical cloud parameters, the isotopic compositions of ice and gas are expected to be in the ranges $\delta^{18}O_{MC} = +100$ to $+250$ per mil (‰) and $\delta^{18}O_{MC} = -60$ to -400‰, respectively, where $\delta^{18}O_{MC} \equiv \{[(^{18}O/^{16}O)/(^{18}O/^{16}O)_{MC}] - 1\} \times 1000$; $(^{18}O/^{16}O)$ and $(^{18}O/^{16}O)_{MC}$ are the isotopic ratios of corresponding chemical species and the bulk molecular cloud (MC), respectively. The degrees of fractionation for calculated $^{18}O/^{16}O$ ratios are consistent with astronomical observations (*9*, *10*). Although the lack of experimental data for $C^{17}O$ predissociation prevents us from a detailed analysis of $^{17}O/^{16}O$ fractionation, its degree is likely near that for $^{18}O/^{16}O$, because the absorption lines of these minor isotope species are unsaturated at least over several tens of A_V (*15*, *16*). Such expected similarity has been recently observed for diffuse interstellar gas (*27*).

In denser and more evolved cold molecular cloud cores, most CO may become frozen onto dust grains. Because of the low temperature, oxygen isotope exchange between CO and H_2O ices is inefficient, and the original isotope fractionation of oxygen is preserved in each phase. Transient external heating by shock waves or by other mechanisms would cause vaporization of both H_2O and CO ices and local homogenization in such a cloud. However, as long as H_2O and CO molecules do not decompose into radicals and atoms, the oxygen isotope fractionation in each molecule is probably preserved.

Here we examine how such isotopic heterogeneity in a molecular cloud may cause the variations of oxygen isotopic compositions observed in our solar system. Taking relative oxygen abundances of silicates, ice, and gas to be 1:2:3 in molecular clouds (*20*), we assumed that both $\delta^{17}O_{MC}$ and $\delta^{18}O_{MC}$ ($\delta^{17\text{ and }18}O_{MC}$) are 0‰ for silicates, +120‰ for ice, and −80‰ for gas (Fig. 1A). The $\delta^{17\text{ and }18}O_{MC}$ values for H_2O ice and CO gas were chosen to be within the simulated ranges and to conserve the mean isotopic composition of the bulk molecular cloud. The ^{16}O-depleted nature of ice relative to silicates is consistent with evidence from a primitive meteorite. The most ^{16}O-depleted known component formed in the solar system is the product of aqueous alteration of Fe,Ni-metal by H_2O in the most primitive ordinary chondrite, Semarkona (*28*).

A protoplanetary disk is formed by collapse of a molecular cloud core. In the outer region of the disk, because of low temperatures (*29*), the primordial oxygen isotopic compositions of the molecular cloud components are preserved (Fig. 1B). CO sublimes while preserving its own oxygen isotope composition outside the orbits of outer planets, even in the case of frozen CO. In the inner region of the disk, H_2O ice evaporates. During an early stage of disk evolution accompanied by vigorous gas accretion, gas-dust fractionation is probably minor, and the mean oxygen isotopic composition of the inner disk gas is reset to the value of the bulk molecular cloud, $\delta^{17\text{ and }18}O_{MC} = 0$‰. Because transient heating events, such as the formation of refractory inclusions and chondrules, were common in the inner solar nebula (*30*), silicate grains would equilibrate with such gas and have similar oxygen isotopic compositions.

As the gas accretion rate decreases, dust-gas fractionation processes begin to proceed in the disk. One such fractionation process is the dust sedimentation toward the disk mid-plane (*31*) (Fig. 1D). In addition, dust particles may preferentially migrate toward the central star (*32*), and ice in the dust evaporates after passing the snow line, releasing ^{16}O-depleted water vapor into the inner disk gas (*33*) (Fig. 1D). This increases the mean $\delta^{17\text{ and }18}O_{MC}$ of disk gas along the mixing line between the oxygen isotopic compositions of CO and of H_2O ice (Fig. 1C), correlating with the degree of H_2O enrichment relative to the H_2O/CO ratio in the parent molecular cloud (*22*). Although enrichment of H_2O by a factor of 10 is justified in the solar nebula (*34*), even moderate enrichment can produce extreme ^{17}O- and ^{18}O-enrichment of the disk gas (Fig. 2). For example, if three times the H_2O enrichment occurs (i.e.,

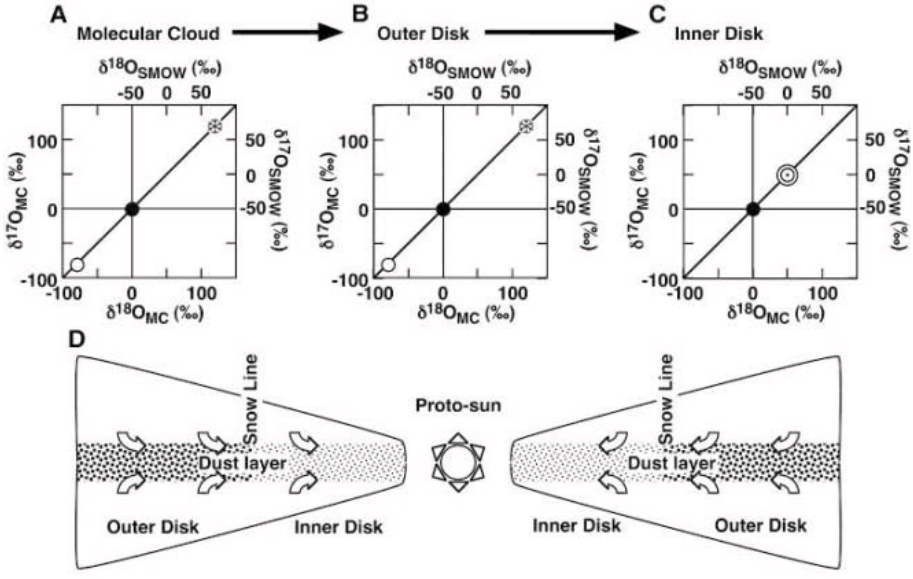

Fig. 1. Schematic diagram of oxygen isotope evolution from a molecular cloud to a protoplanetary disk with dust sedimentation. (**A**) Oxygen isotopic compositions in a molecular cloud. CO (open circle) is the most abundant species next to H_2 and He in a molecular cloud. UV radiation selectively destroys $C^{17\text{ and }18}O$, leaving behind CO enriched in ^{16}O and producing atomic oxygen enriched in ^{17}O and ^{18}O. This heavy oxygen later becomes incorporated into water ice (snowflake). $\delta^{17,18}O_{MC}$ values of 0‰, +120‰, and −80‰ for silicate (solid circles), ice, and gas, respectively, are assumed. (**B**) Oxygen isotopic compositions in the outer disk. The oxygen isotopic composition produced in the molecular cloud is preserved in the individual phases in the outer disk after accretion because of low temperature. (**C**) In the inner disk, oxygen isotopic composition of gas (☉) shifts to a ^{16}O-poor one. (**D**) In the disk, solid materials settle down to the mid-plane and spiral into the proto-sun. Water ice evaporates inside the snow line (*29*), leading to the shift in oxygen isotopic composition shown in (C). Degrees of the shift depend on the H_2O enrichment factor (Fig. 2) and on oxygen isotopic compositions of the individual phases in the molecular cloud. The relationship between δ notation relative to SMOW and that to the molecular cloud possibly corresponds to $\delta^{17\text{ and }18}O_{SMOW} \cong \delta^{17\text{ and }18}O_{MC} - 50$‰, assuming the traditional ^{16}O-rich reservoir in the solar system. The setting of lighter silicates and heavy disk gas with respect to oxygen isotopic composition in the inner disk is consistent with meteoritic observations.

if relative oxygen abundances of ice:gas are 2:1), the mean $\delta^{17\text{ and }18}O_{MC}$ of the inner disk gas will be about +50‰. Therefore, oxygen isotopic compositions of the disk gas are altered easily by dust-gas fractionation processes (*35*). Silicate grains equilibrated with such H_2O-enriched gas during transient heating events would acquire isotope compositions with high $\delta^{17\text{ and }18}O_{MC}$ values (*36*).

Organic materials may also accommodate a significant fraction of oxygen. In hot molecular cloud cores observed for high-mass star-forming regions, CO is probably depleted because of conversion to refractory organics (*37*). Free radical reactions in ices are dominant processes to form refractory organics in molecular clouds (*38*). These organics are interpreted as UV photolysis products in H_2O ice contaminated with CO during the previous cold evolutionary stage of a molecular cloud (*20*). Because oxygen contained in such organics seems to come from H_2O and CO, its isotope composition is expected to be somewhere between those of both species, possibly $\delta^{17\text{ and }18}O_{MC}$ of +20‰ if a 1:1 contribution of H_2O and CO is assumed.

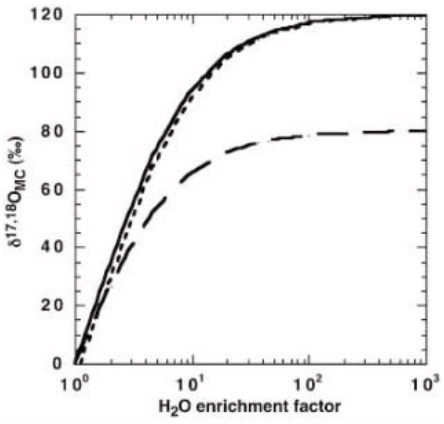

Fig. 2. $\delta^{17\text{ and }18}O_{MC}$ of inner disk gas as a function of the H_2O enrichment factor relative to molecular cloud abundance. The H_2O enrichment factor times the H_2O/CO ratio in a molecular cloud represents the H_2O/CO ratio in the disk gas, excluding chemical equilibria. The solid curve represents a case that neglects refractory organics as an oxygen carrier. Here we assume the $\delta^{17\text{ and }18}O_{MC}$ values of silicate, H_2O ice, and CO gas to be 0‰, +120‰, and −80‰, respectively, and their relative oxygen abundances in a molecular cloud to be 1:2:3. Significant ^{16}O-depletion of the gas is expected even for small H_2O enrichments. Long and short-dashed curves incorporate the effect of organics, providing that the $\delta^{17\text{ and }18}O_{MC}$ values of silicate, ice, organics, and gas are 0‰, +120‰, +20‰, and −80‰, respectively, and that the relative oxygen abundances are 1:1.5:1:2.5 in the molecular cloud. The long-dashed curve indicates the case of higher temperature than the sublimation point of organics, assuming the same enrichment factor with H_2O. The short-dashed curve indicates the case of the temperature between sublimation points of H_2O ice and of organics. In either case, significant ^{16}O-depletion of the gas occurs for small H_2O enrichment factors.

If we accept a refractory organic abundance and composition in a comet nucleus (*20*), the oxygen abundance of organics will be comparable to that of silicate (i.e., silicate:ice:organics:gas = 1:1.5:1:2.5). Because refractory organics evaporate under higher temperatures than H_2O, they may affect the mean oxygen isotopic composition of inner disk gases at high temperatures. Even though this diminishes the amount of change in the isotopic composition because of H_2O enrichment, the disk gas probably has ^{16}O-poor compositions (Fig. 2).

The proposed scenario can reproduce oxygen isotope heterogeneity in the inner solar nebula with an ^{17}O- and ^{18}O-enriched gas, i.e., ^{16}O-depleted gas, relative to silicate dust, consistent with the conventional O isotope reservoirs inferred from meteorite studies (*1*). Under such an environment, the silicate dust evolves into an ^{16}O-depleted composition through isotope exchange with the surrounding gas, because of transient heating events in the nebula. Therefore, the average oxygen isotopic composition of the solar nebula normalized to the standard mean ocean water (SMOW) may be $\delta^{17,18}O_{SMOW} \cong -50‰$ or smaller (*39*) (Fig. 1).

We have shown that even small mass fractionation for CO and atomic O in the molecular cloud can explain the formation of ^{16}O-rich or -poor reservoirs observed for the solar nebula. The ^{16}O-rion or -poor reservoirs can easily form if we use larger mass fractionation factors as expected by chemical models (*16*, *17*) and observations (*27*) of molecular clouds. Thus, the ^{16}O isotope variations may not be unique to our solar system but instead ubiquitous in any planetary system. A direct test of this scenario would be to measure the oxygen isotopic compositions of cometary ices and that of solar wind. We predict the oxygen isotopic values as $\delta^{17\text{ and }18}O_{SMOW} \cong +50$ to +200‰, −100 to −450‰, and −50‰ for cometary H_2O, cometary CO, and solar wind, respectively.

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33. Because of partial support by the radial pressure gradient, disk gas tends to rotate with slightly lower velocity than the Keplerian orbital motion. This causes the frictional loss of angular momentum of immersed solid particles in the disk gas, resulting in their inward migration. The migration speed depends on the particle size and reaches a maximum of ~100 m/s at about a meter in diameter (*32*). The inward radial velocity of gas (v_g) can be estimated from mass accretion rate $\dot{M}$ as $v_g = \dot{M}/(2\pi R\Sigma)$ where R is the distance from the disk center and Σ is the gas column density. Taking $\dot{M} = 10^{-8}$ solar masses per year, which is typical for the disks around classical T-Tauri stars (*40*), and $\Sigma = 3 \times 10^3$ kg/m^3 at $R = 3$ astronomical units of the minimum-mass solar nebula (*41*), $v_g = -0.1$ m/s. In such a disk, the inward radial velocities of solid particles as small as ~1 mm could be several times faster than v_g. Relative motions between the snow line and gas-dust during the disk evolution will also influence the amount of H_2O enrichment in the inner disk. As the disk accretion decays, the snow line would move inward because of disk cooling and in some cases pass the migrating solid particles. However, the snow line cannot enter a warm zone determined by the radiative heating from the central star. Thus, the inward particle migration across the snow line seems to occur unavoidably during disk evolution. Alternatively, H_2O vapor could be depleted from the inner disk if the diffusive outward transport of H_2O vapor and the cold trap outside the snow line are effective (*29*). However, because molecular diffusion is extremely slow, the main mechanism of the outward transport should be the turbulent diffusion. The turbulent flow also causes the inward gas flow because of associated turbulent viscosity. Taking into account the inward gas flow, H_2O vaporized from the solid component migrating inward is eventually supplied into the inner disk. The cold trap mechanism can work only when the solid component is sequestered into bodies

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36. The bulk differences in oxygen isotopic composition observed among the meteorite groups and planets (*1*) could be explained by accretion at different times or by the incorporation of different amounts of nonvaporized water ice and solids with different degrees of the solid-gas equilibration. The solid-gas equilibration and the evolution of oxygen isotopic compositions of the gas in the inner region of the disk may have been recorded by refractory inclusions and chondrules in primitive meteorites.
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39. We use this value according to the traditional ^{16}O-enrichment observed in most Ca-A1–rich inclusions (*1*). Recently, a chondrule having twice the traditional enrichment (δ^{17} and $^{18}O_{SMOW}$ of about –75‰) has been reported (*2*). Although such chondrules are rare, its least fractionated bulk chemical composition from the solar abundance suggests that the average oxygen isotopic composition of silicate in the solar system was originally more enriched in ^{16}O than the traditional value. According to our model, such an extreme ^{16}O-rich chondrule is interpreted as a closer representation of the pristine solar nebula value, implying that the ordinary refractory inclusions are no longer representative of the bulk solar nebula but moderately reprocessed by the interaction with the ^{17}O- and ^{18}O-rich H_2O.
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2 June 2004; accepted 2 August 2004

Middle Miocene Southern Ocean Cooling and Antarctic Cryosphere Expansion

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Magnesium/calcium data from Southern Ocean planktonic foraminifera demonstrate that high-latitude (~55°S) southwest Pacific sea surface temperatures (SSTs) cooled 6° to 7°C during the middle Miocene climate transition (14.2 to 13.8 million years ago). Stepwise surface cooling is paced by eccentricity forcing and precedes Antarctic cryosphere expansion by ~60 thousand years, suggesting the involvement of additional feedbacks during this interval of inferred low-atmospheric partial pressure of CO_2 (pCO_2). Comparing SSTs and global carbon cycling proxies challenges the notion that episodic pCO_2 drawdown drove this major Cenozoic climate transition. SST, salinity, and ice-volume trends suggest instead that orbitally paced ocean circulation changes altered meridional heat/vapor transport, triggering ice growth and global cooling.

The middle Miocene climate transition (MMCT), 14.2 to 13.8 million years ago (Ma), is one of the three major steps in Earth's Cenozoic climate evolution (*1*–*3*). The ~1‰ increase in the oxygen-isotopic composition ($\delta^{18}O$) of benthic foraminifera describes a combination of Antarctic ice growth and global cooling at ~14 Ma, as is also indicated by Southern Ocean ice-rafted detritus, eustatic change, and the fossil record (*1*–*6*). However, because $\delta^{18}O$ records both temperature and global ice volume, fundamental questions and uncertainties exist concerning the magnitude and phasing of middle Miocene ice growth and cooling. The development of Mg/Ca, an independent paleotemperature proxy measured on the same foraminiferal calcite ($CaCO_3$) as $\delta^{18}O$, has facilitated isolation of the ice-volume component of $\delta^{18}O$ records (*7*–*12*). The Mg/Ca content of foraminifera increases exponentially with temperature (~9 ± 1% per 1°C) and is relatively insensitive to salinity and ice-volume fluctuations (*7*, *8*). Low-resolution paired benthic foraminifer Mg/Ca and $\delta^{18}O$ studies designed to constrain the timing and magnitude of pre-Quaternary ice-volume fluctuations suggest substantial Antarctic ice growth (~0.85‰) and a concomitant deep ocean cooling (2°C to 3°C) during the MMCT (*11*, *12*). The magnitude of Antarctic ice growth and rapidity of this climate transition [<0.5 million years (My)] suggests that Earth's climate system was highly sensitive to oceanic, atmospheric, and cryospheric feedbacks.

Ocean circulation and atmospheric pCO_2 variations are often cited as potential catalysts of the MMCT (*13*–*17*). Large-scale reorganizations of ocean circulation driven by atmospheric circulation changes and/or tectonic reorganizations of gateway regions may have altered poleward heat and moisture transport, resulting in Antarctic ice growth and global cooling (*13*–*15*). Ocean circulation hypotheses are supported by $\delta^{13}C$ proxy evidence (*14*, *15*, *18*, *19*) and the timing of tectonic events in the eastern Tethys/Indonesia (*4*, *20*) and the North Atlantic (*13*). Alternatively, atmospheric pCO_2 drawdown, through organic carbon sequestration on the mid-latitude continental margins (*16*) and/or enhanced silicate weathering rates (*17*), may have driven Antarctic ice-sheet expansion and cooling at ~14 Ma. Support for this "Monterey Hypothesis" comes from thick, organic carbon-rich Miocene sedimentary sequences around the Pacific Rim (*4*, *16*) and a corresponding ~ 1‰ increase in global deep sea $\delta^{13}C$ (*4*, *16*, *21*, *22*). A potential complication of the hypothesis is revealed by paleo-pCO_2 estimates (*23*–*25*), which indicate that atmospheric pCO_2 levels declined >3 My before the MMCT and provide little support for either elevated atmospheric pCO_2 during the warm Miocene climatic optimum (MCO) (17 to 14 Ma) or a semipermanent atmospheric pCO_2 decrease at the MMCT. These estimates indicate that factors other than those related to global carbon cycling may contribute to this major Cenozoic climate transition. To evaluate the processes and feedbacks involved in the MMCT, detailed information is needed regarding the phasing of carbon cycling, Antarctic ice growth, and high-latitude oceanic/atmospheric cooling. Acquiring this information has thus far proven difficult because of the limited availability of $CaCO_3$-rich Southern Ocean sediments and the lack of an unambiguous paleotemperature proxy.

Here, we present an independent record of middle Miocene high-latitude Southern Ocean sea surface temperature (SST). To establish the thermal and hydrographic response of Southern Ocean surface waters and the phasing of high-latitude SST change, Antarctic cryosphere expansion, and global carbon cycling between ~17 and 13 Ma, we

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generated paired Mg/Ca and $\delta^{18}O$ records from surface dwelling planktonic foraminifer *Globigerina bulloides* in conjunction with benthic foraminifer (*Cibicidoides mundulus*) $\delta^{18}O$ and $\delta^{13}C$ records at Ocean Drilling Program (ODP) Holes 1170A (47°90'S, 146°02.98'E; 2704 m) and 1171C (48°30'S, 149°06.69'E; 2150 m) on the South Tasman Rise (STR) (*26*) (Fig. 1). Plate tectonic reconstructions indicate that Sites 1170 and 1171 were situated at ~55°S in the middle Miocene (*27*), with calculated paleodepths of 2100 m and 1600 m, respectively (*28*). We used *G. bulloides* for several reasons. First, a well-defined modern subantarctic *G. bulloides* Mg-temperature calibration exists (*7*). Second, previous studies demonstrate the utility of *G. bulloides* Mg/Ca in reconstructing Quaternary subantarctic SSTs (*7*, *9*). Finally, *G. bulloides* is continuously present through both STR middle Miocene sequences (*26*). Hole 1171C Miocene *G. bulloides* Mg/Ca ranges between ~1.7 and 4.3 mmol/mol (Fig. 2 and table S1); minimum Mg/Ca values are similar to those from subantarctic core tops (*7*, *9*). The main feature of the *G. bulloides* Mg/Ca record is the 1.8 mmol/mol point-to-point decrease centered at 166.4 m below sea floor (mbsf) that marks the transition from relatively high to relatively low Mg/Ca. Three step-like features are superimposed on this transition (Fig. 2). Hole 1171C *G. bulloides* $\delta^{18}O$ ranges between ~0.0‰ and ~1.8‰ (*29*) (Fig. 2 and table S1). The main feature of the *G. bulloides* $\delta^{18}O$ record is the 0.9‰ point-to-point increase centered at 165.6 mbsf that marks the transition to more positive $\delta^{18}O$ values. The step-like structure of the Mg/Ca transition does not appear in the *G. bulloides* $\delta^{18}O$ record, which reflects ice volume and salinity influences in addition to temperature.

Diagenesis and dissolution may potentially alter the primary Mg/Ca signal encoded in planktonic foraminiferal calcite and, thus, the inferred SST record (*8*–*11*) (SOM Text). Several lines of evidence suggest that these processes have not biased our STR middle Miocene Mg/Ca values: (i) *G. bulloides* ultrastructures are visible, and shells do not exhibit extensive pitting, fragmentation, infilling, or overgrowth; (ii) the average $CaCO_3$ content of the middle Miocene ooze is stable at ~94% by weight (*26*) as a result of the intermediate STR paleodepths (*28*); and (iii) benthic and planktonic foraminifers exhibit interspecific $\delta^{18}O$ and $\delta^{13}C$ offsets (*30*).

We converted STR *G. bulloides* Mg/Ca values to temperatures using the calibration of Mashiotta *et al*. (*7*): SST = ln(Mg/Ca/0.474)/0.107 (SE, ±0.8°C). This calibration successfully estimates subantarctic Pacific (45°S to 56°S) and Indian Ocean (43°S) austral spring SSTs in both the modern and Quaternary (*7*, *9*). As ours is the first pre-Quaternary study employing this calibration, we assume that the environmental preferences of middle Miocene subantarctic *G. bulloides* and physical processes governing Mg uptake are similar to those in modern subantarctic *G. bulloides*. These processes and seawater Mg/Ca (*31*) may have changed through time and could contribute an additional uncertainty of up to 3°C in the conversion of Mg/Ca to absolute temperature (SOM Text). However, this uncertainty would not affect the magnitude of inferred temperature shifts on time scales <1 My. Age models derived from magnetostratigraphy, biostratigraphy, and stable isotope datums (*32*) provide the chronologic framework for the STR records (SOM Text).

Mg/Ca evidence from Hole 1171C indicates that regional SSTs were ~2°C cooler after ~14 Ma (14.7 ± 1.1°C; 13.9 to 12 Ma) than during the preceding MCO (17.0 ± 1.2°C; 17 to 14 Ma) (Fig. 3) (*3*, *4*). Paleobotanical and faunal paleotemperature estimates from southern Australia and New Zealand support this trend and are similar to, or slightly cooler than, *G. bulloides* Mg/Ca-derived SST estimates (*4*, *33*). The major feature of the STR SST record is a ~7°C (point-to-point) transition (14.2 to 13.9 Ma) from the warmest MCO to cooler post-MMCT SSTs

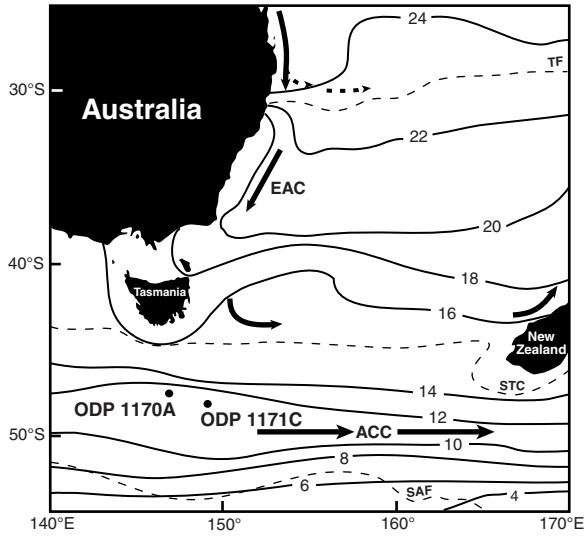

Fig. 1. Annual average SSTs in the subantarctic southwest Pacific (*39*) with Ocean Drilling Program (ODP) South Tasman Rise (STR) sites indicated. ODP Site 1171 is located at the intersection of the southward-flowing East Australian Current (EAC) and the eastward-flowing Antarctic Circumpolar Current (ACC), one of three locations where heat is introduced to the Southern Ocean (*26*). ODP Site 1170 is located 50 km northwest of Site 1171, beyond the influence of the EAC (*26*). In the middle Miocene, the STR was situated at ~55°S, 5° to 7° south of its present location (*27*). Backtracked middle Miocene paleodepths of Sites 1170 and 1171 are 2100 m and 1600 m, respectively (*28*). Currently, STR hydrography is controlled by the Subtropical Convergence (STC) to the north and the Subantarctic Front (SAF) to the south. Seasonal SST variation is ~4°C (*26*, *39*). *G. bulloides* calcifies in the mixed layer during the austral spring when regional surface temperatures range between 8°C and 12°C (*9*, *39*).

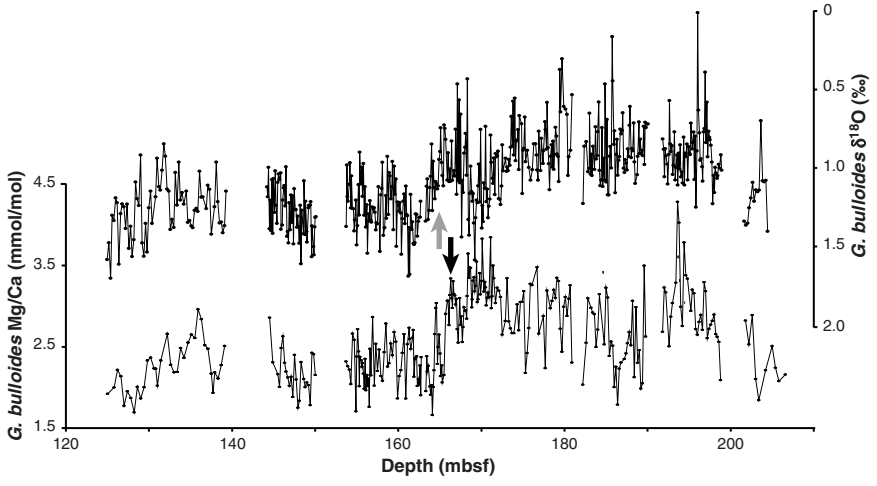

Fig. 2. Climate proxy data from South Tasman Rise (STR) ODP Hole 1171C (48°30'S, 149°06.69'E; 2150 m). The Mg/Ca record was generated on planktonic foraminifer species *G. bulloides* at 20-cm intervals from 125 to 154 mbsf and from 154 to 207 mbsf and at 10-cm intervals between 154 and 172 mbsf. Each point represents an average of one to four analyses; pooled SD of all replicates (df = 64) is ±0.21 mmol/mol (±7.8%). Measured modern southwest Pacific core top Mg/Ca is ~1.6 mmol/mol (*9*). Oxygen isotope ($\delta^{18}O$) data were generated from *G. bulloides* at 10-cm intervals between 125 and 207 mbsf. The black arrow denotes the midpoint of the major 1.8 mmol/mol Mg/Ca transition, and the gray arrow denotes the midpoint of the 0.9‰ *G. bulloides* $\delta^{18}O$ transition. Gaps in the data are due to incomplete core recovery (*26*).

(Fig. 3). Inferred orbital-scale [~400 and 100 thousand years (ky)] SST variability is particularly pronounced during the MMCT. Cooling occurred in three distinct steps (midpoints, 14.2, 14.0, and 13.9 Ma) and was followed by an interval of relatively cold SSTs (13.7 ± 1.1°C; 13.9 to 13.8 Ma) (Figs. 3 and 4). At ~13.8 Ma, regional SSTs warm by ~2°C to 3°C and remain relatively stable until ~12 Ma. We confirmed the Hole 1171C SST estimates by measuring *G. bulloides* Mg/Ca at a second STR location, Hole 1170A [~54°S (*27*); 2100 m (*28*)] (Figs. 1 and 3, table S2), with a different depositional history (*26*). Similarities in both magnitude (±0.5°C) and structure of the two records (Fig. 3) further support our interpretation of STR *G. bulloides* Mg/Ca as a primary climate signal. We attribute several offsets between the records to age model and sampling resolution differences (*30*) (SOM Text).

Large-amplitude SST fluctuations over the STR suggest substantial reorganization of Southern Ocean surface waters during the MMCT. One possible explanation is an orbitally paced intensification of the Antarctic Circumpolar Current (ACC) system, perhaps related to increased westerly wind strength. This idea is supported by a paleobotanic record of regional wind strength (*4*, *34*), substantial evidence for increased meridional thermal gradients (*4*), the timing of major Southern Ocean unconformities (*4*, *5*), and STR surface water $\delta^{18}O$ ($\delta^{18}O_w$). We used Hole 1171C *G. bulloides* Mg/Ca SST and $\delta^{18}O$ (table S1) to calculate $\delta^{18}O_w$ (*7*, *8*) (SOM Text) (fig. S1), which should reflect some combination of ice volume and local salinity effects. Large SST fluctuations during the MMCT obscure the *G. bulloides* $\delta^{18}O$ increase, which indicates that STR $\delta^{18}O_w$ likely reflects regional salinity variations. Strict interpretation of the $\delta^{18}O_w$ results suggests a gradual freshening of regional surface waters between 14.2 and 13.8 Ma, consistent with enhanced ACC strength and the resultant increased influence of subantarctic surface waters over the STR. A notably similar pattern of oceanographic change is recognized in the southwest Pacific during Quaternary glaciations (*9*, *35*).

Orbital-scale cyclicity (400 and 100 ky) is inferred in the STR records of *G. bulloides* Mg/Ca and *C. mundulus* $\delta^{18}O$ and $\delta^{13}C$. This cyclicity indicates a potential role for Milankovitch forcing during the MMCT, which suggests that Earth's climate system was particularly sensitive to forcing at eccentricity frequencies between ~15 and 13.5 Ma. Comparison of orbital curves (*36*) to untuned STR SST and *C. mundulus* $\delta^{18}O$, an ice-volume proxy (*32*) (SOM Text), reveals that the MMCT occurred within an interval of high obliquity and moderate eccentricity variance (Fig. 4) (*36*). This relationship suggests that, at 14.2 Ma, Antarctic ice sheets were large enough to survive warm summer orbital configurations but small enough to respond dynamically to orbital forcing (*37*). Surface cooling generally corresponds with long-period (~400 ky) eccentricity minima between 14.2 and 13.8 Ma (which are similar in amplitude to previous and subsequent minima) and precedes Antarctic ice growth by ~60 ky (as judged by the midpoints of the major Mg/Ca and *C. mundulus* $\delta^{18}O$ transitions). Our data suggest that orbital forcing paced both Southern Ocean SSTs and Antarctic ice growth during the MMCT. There is, however, no obvious orbital anomaly akin to that recognized at the Oligocene/Miocene boundary (*38*), and, assuming a synchronous atmospheric and SST response, the substantial time lag (~60 ky) between orbital forcing and ice growth supports the notion that additional feedbacks (e.g., carbon cycling, ocean circulation, and/or ice/albedo feedbacks) may be required for substantial and rapid Antarctic ice growth. Heightened sensitivity to eccentricity forcing between 14.2 and 13.8 Ma and the phasing of SST and ice volume ($\delta^{18}O$) are clues to identifying the feedbacks involved in this climate threshold.

Model results stress a fundamental role for atmospheric pCO_2 in Cenozoic climate change and indicate that the Antarctic cryosphere may react sensitively to climate feedbacks only when atmospheric pCO_2 is relatively low and within some narrowly defined

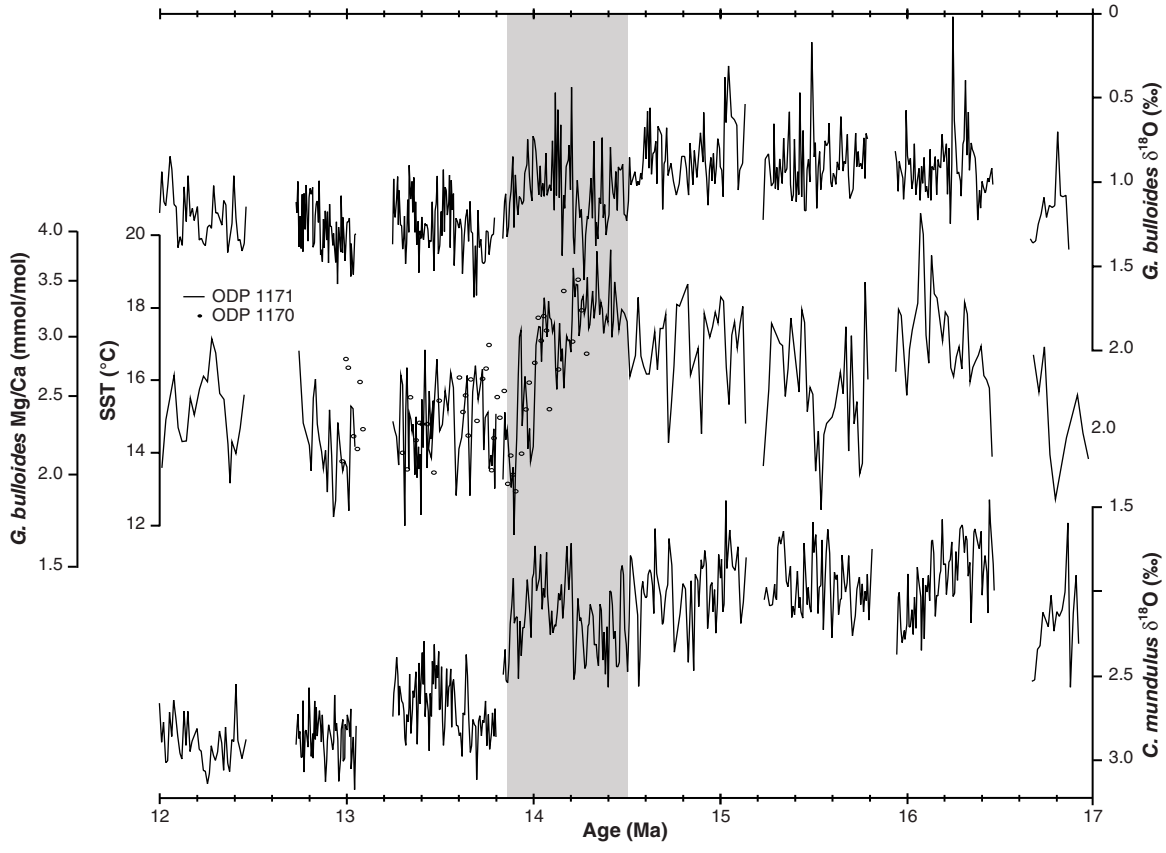

Fig. 3. Hole 1171C middle Miocene (12 to 17 Ma) *G. bulloides* $\delta^{18}O$, SST, and benthic foraminifer (*C. mundulus*) $\delta^{18}O$ (*32*) records versus age. Mg/Ca–derived SSTs from Hole 1170A (open circles) are plotted with the Site 1170 age model. Age models at Sites 1170 and 1171 were constructed using magnetostratigraphy, biostratigraphy, and stable isotope datums (*26*, *32*). The *G. bulloides* Mg/Ca values were converted to SST by using the equation of Mashiotta *et al*. (*7*). Both the Mg/Ca and SST scales are given. The *C. mundulus* $\delta^{18}O$ record serves as a general proxy for Antarctic ice volume (*11*, *30*) (SOM Text).

range (*37*). Sensitivity to eccentricity forcing is apparent in many middle Miocene $\delta^{13}C$ records between 17 and 13.5 Ma (*4*, *14*, *15*, *18*, *21*, *22*), which suggests at least some role for the carbon cycle in the MMCT. However, the >3 My discrepancy between the initial atmospheric pCO_2 decline (*23*, *24*) and the MMCT has shifted focus to the role of episodic pCO_2 drawdown, associated with more positive $\delta^{13}C$ carbon maxima (CM) events, in triggering this major climate step (*21*). Moderate resolution records suggest that CM events, which are attributed to climate-induced variations in marine organic carbon sequestration, co-vary with analogous $\delta^{18}O$ events at the 400-ky frequency (*14*, *15*, *18*). Comparison of STR *G. bulloides* Mg/Ca to *C. mundulus* $\delta^{18}O$ (ice volume) and $\delta^{13}C$, a general proxy for carbon cycling and atmospheric pCO_2, reveals that Southern Ocean surface-water cooling and Antarctic ice growth occurred between CM5 and CM6 during an interval of increasing pCO_2 (Fig. 4) (*23*). This sequence of change challenges the notion that episodic pCO_2 drawdown was the primary forcing that triggered the MMCT, again implying that other feedbacks (e.g., ice/albedo) played a more significant role in this climate transition. Our records also indicate that CM6, one of the largest CM events, corresponds with maximum Antarctic ice volume and a slight warming of the high southern latitudes. This relationship raises the possibility that feedbacks related to Antarctic cryosphere expansion may have exerted control over the global carbon cycle through enhanced ventilation of intermediate and deep ocean waters and falling sea levels (*4*, *6*, *23*). The long-term trend observed in the global $\delta^{13}C$ record after the rapid expansion of Antarctic ice volume supports this interpretation (*3*).

Our results demonstrate that STR SSTs cooled 6°C to 7°C between 14.2 and 13.8 Ma, revealing that the Southern Ocean was a dynamic component of the MMCT. Eccentricity-paced Southern Ocean surface cooling and freshening suggest that atmospheric/oceanic circumpolar circulation intensified in response to orbital forcing, increasingly thermally isolating Antarctica during the MMCT (14.2 to13.8 Ma). Middle Miocene intensification of the ACC may have played a major role in Cenozoic climate evolution, both directly through changes in meridional heat transport and indirectly through changes in vapor transport. We speculate that sensitivity to eccentricity forcing increased at 14.2 Ma, immediately following peak warmth of the MCO (*23*–*25*) as a result both of low atmospheric pCO_2 (*37*) and of a fundamental reorganization of the climate system, specifically a tectonically mediated reduction in meridional heat/vapor transport related to the constriction of the Eastern Tethys at ~15 Ma (*4*, *14*, *20*). The presence of 100-ky variability (14.2 to 13.8 Ma) and a rare shift in eccentricity cadence between 15 and 14 Ma (*36*) are intriguing, and future efforts should focus on understanding the evolution of the climate spectrum on orbital time scales during the MMCT.

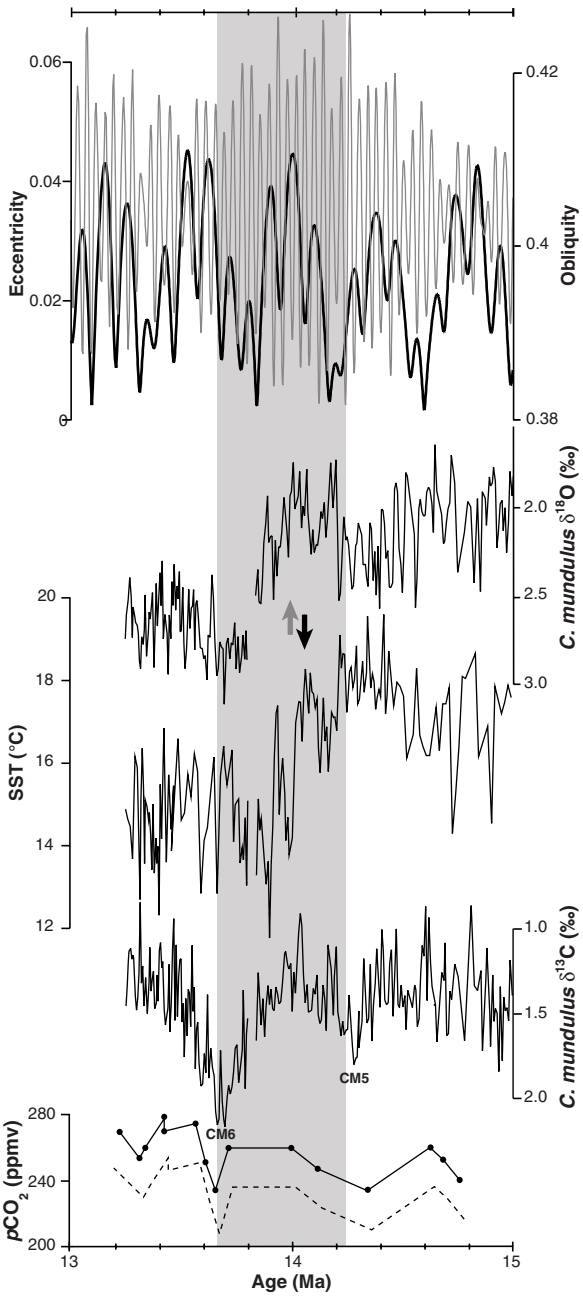

Fig. 4. Expanded view of the 13- to 15-Ma interval. Site 1171 SSTs are compared with *C. mundulus* $\delta^{18}O$ and $\delta^{13}C$ records (*32*). Orbital solutions (*36*) are plotted; eccentricity (black) and obliquity (gray). Paleo-pCO_2 estimates derived from southwest Pacific alkenone $\delta^{13}C$ (*23*); maximum (solid line) and minimum (dashed line) estimates are plotted. All records are plotted on the Site 1171 age scale (*32*) (SOM Text). Midpoints of the SST and *C. mundulus* $\delta^{18}O$ transitions are 14.07 Ma (black arrow) and 14.01 Ma (gray arrow), respectively. The final $\delta^{13}C$ increase of the "Monterey" $\delta^{13}C$ excursion (CM6) begins at 13.8 Ma and terminates at 13.6 Ma.

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40. Supported by NSF grant OPP0229898 to J. P. Kennett and JOI/USSSP postcruise funds to A. E. Shevenell. This research used samples provided by the Ocean Drilling Program. Mass spectrometer operation/support by G. Paradis and H. Berg and laboratory assistance from K. Thompson were critical to the success of this study. We thank the Gulf Coast Repository staff for providing samples; I. Hendy, M. Hommeyer, P. Martin, and D. Pak for discussion; and reviewers for suggestions.

Supporting Online Material

www.sciencemag.org/cgi/content/full/305/5691/1766/DC1
Materials and Methods
SOM Text
Fig. S1
Tables S1 and S2
References

7 May 2004; accepted 10 August 2004

Crystal Structure of a Shark Single-Domain Antibody V Region in Complex with Lysozyme

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Cartilaginous fish are the phylogenetically oldest living organisms known to possess components of the vertebrate adaptive immune system. Key to their immune response are heavy-chain, homodimeric immunoglobulins called new antigen receptors (IgNARs), in which the variable (V) domains recognize antigens with only a single immunoglobulin domain, akin to camelid heavy-chain V domains. The 1.45 angstrom resolution crystal structure of the type I IgNAR V domain in complex with hen egg-white lysozyme (HEL) reveals a minimal antigen-binding domain that contains only two of the three conventional complementarity-determining regions but still binds HEL with nanomolar affinity by means of a binding interface comparable in size to conventional antibodies.

The cartilaginous fish (sharks, skates, rays, and chimeras) diverged from a common ancestor with other jawed vertebrates approximately 500 million years ago and include over 700 extant species. Nevertheless, they possess an adaptive immune system based on immunoglobulin (Ig), T cell receptors (TCRs), and the major histocompatibility complex. Three immunoglobulin isotypes have been identified in cartilaginous fish: two standard heavy (H)-light (L)-chain isotypes, designated IgM and IgW (IgW is also called IgX or IgNARC); and an atypical isotype, IgNAR. IgNAR is an H-chain homodimer that does not associate with L chain (*1*, *2*), unlike conventional human and murine antibodies (Fig. 1). Each H chain has one variable (V) and five constant (C) domains. Electron microscopy studies of IgNAR have revealed that their V regions are single domains, tethered to the C domains by means of flexible hinge-like regions (*2*). The V regions of IgNAR are not closely related to the V_H regions of either shark IgM or IgW in phylogenetic tree analyses; rather, they cluster with the V regions of TCR or immunoglobulin L chains (*1*, *3*).

In addition to the two canonical cysteines typical of immunoglobulin domains, IgNAR V domains carry a number of noncanonical cysteines that enable classification into two closely related subtypes, I and II. Type II V regions have an additional cysteine in complementarity-determining regions (CDRs) 1 and 3, which have been proposed to form a domain-constraining disulfide bond, akin to those observed in camelid H-chain V (V_HH) domains (*2*, *4*), whereas the extra cysteines in type I V regions are in framework regions (FRs) 2 and 4, and another two or four cysteines are

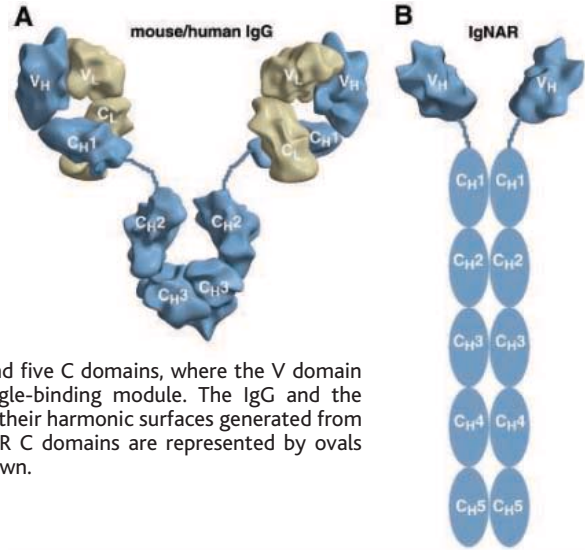

Fig. 1. Schematic representation of the overall IgG and IgNAR architectures. (**A**) A conventional IgG is composed of two H chains (blue) and two L chains (yellow) that assemble to form one Fc and two Fab regions or superdomains. The H chain has three C domains (C_H1, C_H2, and C_H3) and one V domain (V_H), whereas the L chain has one C domain (C_L) and one V domain (V_L). The V region is made up of two immunoglobulin domains (V_H and V_L). (**B**) IgNAR has only two H chains, each consisting of one V and five C domains, where the V domain is unpaired and constitutes a single-binding module. The IgG and the IgNAR domains are represented by their harmonic surfaces generated from atomic coordinates (*27*); the IgNAR C domains are represented by ovals because their structures are unknown.

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in CDR3. These CDR3 cysteines are usually encoded by the diversity (D) regions in their preferred reading frame (*2*) and are under strong selection in the primary repertoire (*5*). A deletion in the FR2-CDR2 region gives the IgNAR V domain its characteristically small size (~12 kD). Furthermore, somatic mutations that are found in the CDR1 of type II and in the shortened FR2-CDR2 region of type I appear to correlate with the acquisition and particular location of these extra disulfides in shark V regions (*5*).

IgNAR genes are found in the "cluster configuration" typical of cartilaginous fish immunoglobulins (*6*), with each cluster containing a single V region, three diversity elements, and a single joining gene (*1*). Because rearrangement only occurs within a cluster (*1*, *6*–*8*) and only a single functional cluster is present for each IgNAR type I or type II (*1*, *9*), diversity encoded by the V germline genes is severely limited. However, the repertoire is expanded greatly through the generation of enormous diversity in CDR3 through four rearrangement events, which include nucleotide (N)–region additions at each joining region, and by a high rate of antigen-driven somatic hypermutation (*10*).

Dimeric H-chain antibodies are also present in camels and llamas, where each H chain contains one V_HH domain and two C domains. Unlike IgNAR H chains, which are not known to have ever associated with L chains, camel H-chain antibodies have lost their ability to associate with L chain as a result of a deletion of their C_H1 domains and modification of V_H residues that would normally interact with V_L in conventional antibodies (*11*). Crystal and nuclear magnetic resonance structures for camel and llama V_HH single domains (*12*–*24*) have inspired antibody engineering of single-domain antigen-binding fragments for use in biotechnology and medicine (*25*).

The IgNAR V region clone HEL-5A7 was selected from a phage-displayed library derived from a nurse shark (*Ginglymostoma cirratum*) immunized with HEL. The HEL-5A7 single domain is highly stable, highly specific, and binds HEL with nanomolar affinity (*26*). The crystal structure of the IgNAR HEL-5A7 at 1.45 Å resolution is presented here (*27*) (Fig. 2 and table S1). Comparison of this shark IgNAR V domain with antibody and TCR immunoglobulin domains from higher vertebrates (Fig. 3) now permits more informed speculation about the origins and evolution of these immunologically important antigen-binding receptors.

The IgNAR V domain has an immunoglobulin β sandwich fold (Figs. 2A and 3A) consisting of only 8 β strands, rather than the 10 in a conventional antibody or TCR V domain, because of the deletion of the C′ and C′′ strands that normally comprise CDR2 (Fig. 3). As in C_L domains, the C strand connects directly to the D strand (Fig. 3, A and D). The CDR3 loop is long (*28*) compared with most human and especially murine CDR H3s, but it is of average length for IgNAR type I V regions [15 to 27 residues (*5*, *26*)]. The type I IgNAR CDR3 contains unusual disulfide bonds [N35 (FR2) to N92 (CDR3) and N97 (CDR3) to N104 (FR4)] (Fig. 2A) that constrain the CDR3 loop to fold over the outside sheet that would usually associate with V_L (IgNAR and lysozyme residues are designated by N- and L-chain identifiers, respectively). CDR3 residues N93 to N99 form a 3_{10} helix, similar to CDR3 conformations in several camelid V_HH domains.

The IgNAR V domain shares features with several types of vertebrate immunoglobulin domains, making it difficult to classify. Sequence homology is highest (~35% identity) between IgNAR V and TCR V_α or immunoglobulin V_κ chains, whereas structural homology is greatest with V_α, V_L, and V_H domains (*29*). From a gross topological comparison, IgNAR V (Fig. 3A) is similar to a C1-type domain (Fig. 3D), which does not have C′ and C″ strands. However, as in conventional V-type domains, the IgNAR A-strand hydrogen bonds to both "top" and "bottom" sheets, by means of a bend at Pro^{N7}. Strand A most resembles A strands in V_κ (Fig. 3B) and V_α (Fig. 3C) domains by

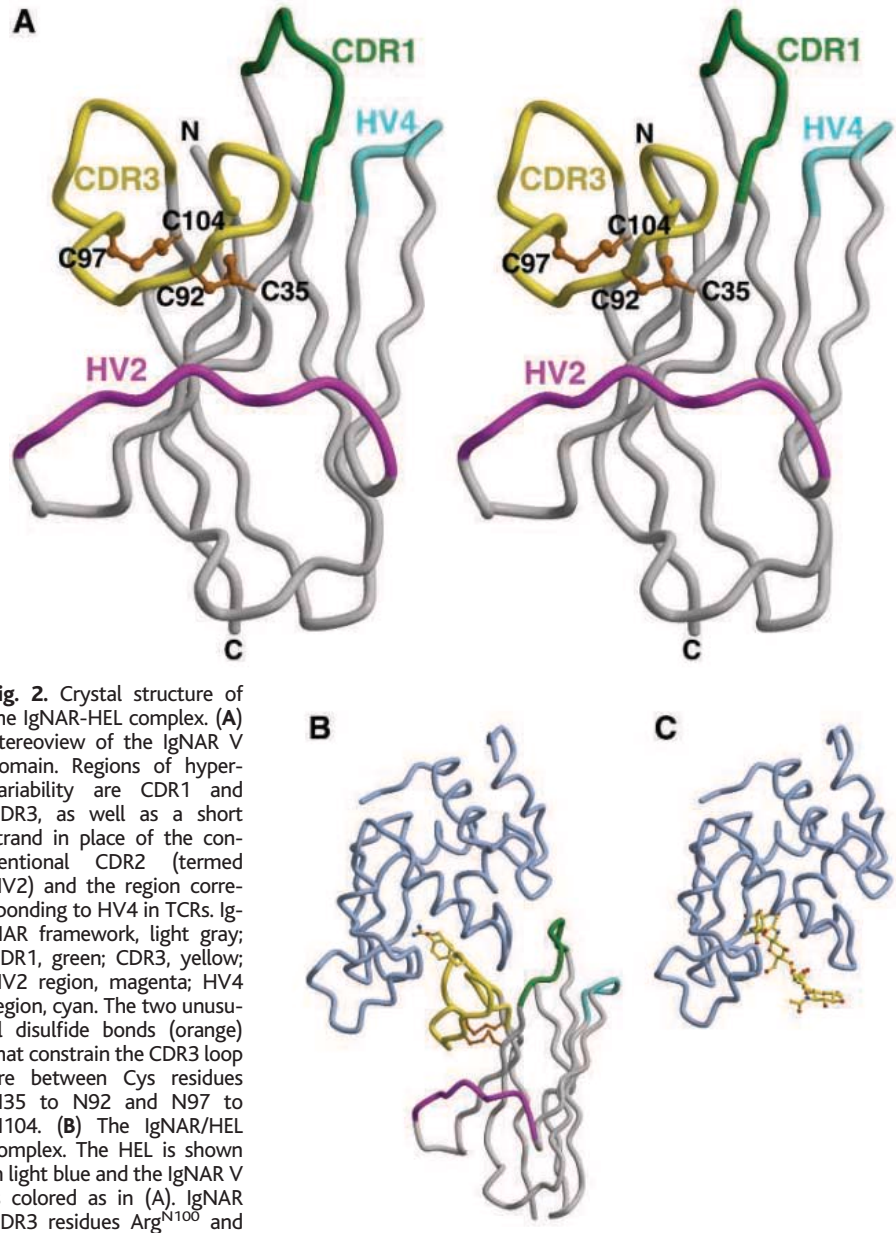

Fig. 2. Crystal structure of the IgNAR-HEL complex. (**A**) Stereoview of the IgNAR V domain. Regions of hypervariability are CDR1 and CDR3, as well as a short strand in place of the conventional CDR2 (termed HV2) and the region corresponding to HV4 in TCRs. IgNAR framework, light gray; CDR1, green; CDR3, yellow; HV2 region, magenta; HV4 region, cyan. The two unusual disulfide bonds (orange) that constrain the CDR3 loop are between Cys residues N35 to N92 and N97 to N104. (**B**) The IgNAR/HEL complex. The HEL is shown in light blue and the IgNAR V is colored as in (A). IgNAR CDR3 residues Arg^{N100} and Tyr^{N101} are deeply buried in the HEL active site. (**C**) The crystal structure of the HEL $Asp^{52}\rightarrow Ser^{52}$ mutant in complex with oligosaccharide [PDB accession code 1LSZ (*27*)] highlights the location of the lysozyme active site. The lysozyme is oriented as in (B).

length (one residue shorter than V_λ and V_H) and in the cis conformation of Pro^{N7} (highly conserved in V_κ domains). This structural homology with V_α or V_κ domains reflects the previous clustering of IgNAR V sequences with V regions of TCR and immunoglobulin L chains during the original phylogenetic analysis (*1*).

With the exception of CDR H3, the CDR loops of human and murine antibodies generally have "canonical" structures (*30*). However, the IgNAR CDR1 loop does not closely resemble human or murine canonical CDR1s (Fig. 3, A, B, and E) but instead converges on the "type 4" CDR1 conformation found in two camel V_HH domains, cAb-Lys3 and cAb-RN05 (*31*) (figs. S1 and S2). This similarity suggests that its conformation may be influenced by structural features and functional requirements specific to single-domain antibodies.

The IgNAR V region only contacts HEL by means of CDR1 and CDR3, with CDR3 residues inserted directly into the HEL active-site cleft (Figs. 2, B and C, and 4; tables S2 to S4). IgNAR Arg^{N100} forms a salt bridge with the catalytic Asp^{L52}, which explains its partial inhibition of HEL enzymatic activity (*9*) and lack of cross-reactivity with turkey egg-white lysozyme (*26*, *32*). The molecular surface area buried in the complex is extensive, considering that only two CDRs are used for antigen interaction (*33*), with 666 and 734 $Å^2$ of surface buried on IgNAR V and HEL, respectively (*34*).

A model was constructed of the IgNAR type II V domain, primarily on the basis of this type I V region structure (fig. S3) (*35*), which supports a proposed noncanonical disulfide between CDR3 and CDR1. The type II CDR3 would then adopt a more extended conformation and protrude from the antibody framework akin to the camelid V_HH AMD10 CDR3 (fig. S3). Both the IgNAR Type II model and AMD10 have an exposed lysine at the structurally equivalent positions 84 (IgNAR) or 96 (AMD10) (*36*) rather than a Gly, as in IgNAR Type I and most other camelid V_HH structures. Glycines at this position are not solvent exposed, but instead are covered by the long CDR3 that folds over and masks this hydrophobic surface (the V_H/V_L interface in conventional antibodies).

In a study of random IgNAR V cDNAs (*5*), a number of residues were identified as having either high (positive selection) or low (negative selection) ratios of replacement to silent mutations. In addition to the CDR1 and CDR3 regions, residues in hypervariable region 2 (HV2) (N45 to N51) were found to be under strong positive selection (*37*). Their hypermutation among different IgNAR V regions could be either because of the direct recognition of the antigen [as observed in camelid domains AMB7 and AMD10 (*20*)] or because they maintain and influence the conformation of the CDR3, which does directly contact the antigen (supporting online material text).

Some species of shark respond readily to foreign antigens with a potent IgNAR response (*9*), and several libraries of IgNAR V regions displayed in bacteriophage have already been constructed (*26*, *38*, *39*), which should facilitate engineering of high affinity, minimal antigen-binding domains for biotechnological and biomedical use. The type I V region structure and type II model presented here suggest that these single-domain shark antibodies can generate sufficient structural diversity to recognize a wide array of antigens by varying the positions of their germline-encoded cysteines, which then markedly affect the conformation of CDR3 and the binding-site architecture. Whether this single-domain antibody did indeed evolve from a primordial antigen-binding receptor or whether it was derived later in phylogeny will remain unresolved until IgNAR (or IgNAR-like) molecules are found in more phylogenetically distant vertebrates, such as the jawless vertebrates, lamprey (*40*, *41*), and hagfish.

Note added in proof: While this paper was in production, a paper reporting the structures of the two unliganded type 2 IgNAR variable domains was published (*42*).

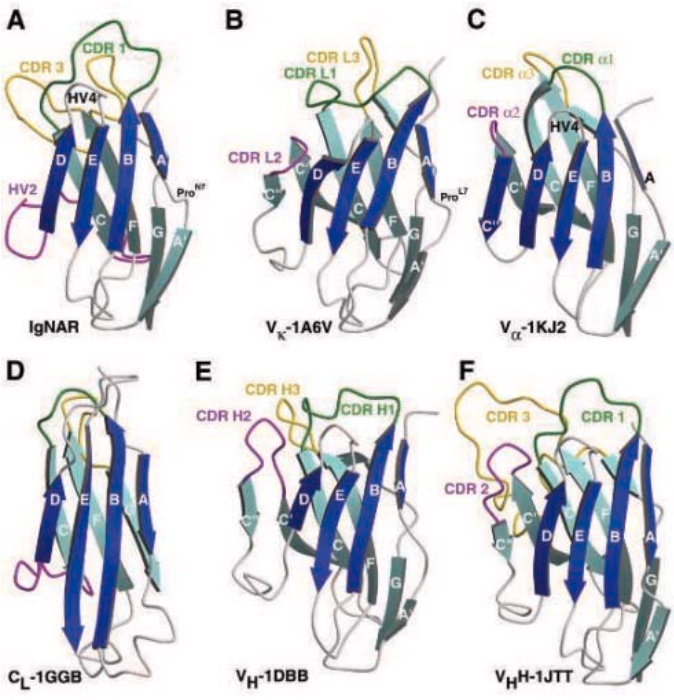

Fig. 3. Comparison of the IgNAR V domain with other immunoglobulin domains. (**A**) In IgNAR, the front sheet consists of strands A, B, E, and D, whereas the back sheet consists of strands A′, G, F, and C. The bend between A and A′ is similar to that seen in most V_κ domains, with a cis-Pro residue at position N7. (**B**) IgG V_κ domain from Fv B1-8 (PDB accession code 1A6V). (**C**) TCR V_α domain from TCR KB5-C20 (PDB accession code 1KJ2). (**D**) IgG C_L chain domain from Fab 50.1 (PDB accession code 1GGB). (**E**) IgG V_H domain from Fab DB3 (PDB accession code 1DBB) (**F**) Camel V_HH domain from cAb-Lys3 (PDB accession code 1JTT) (*27*).

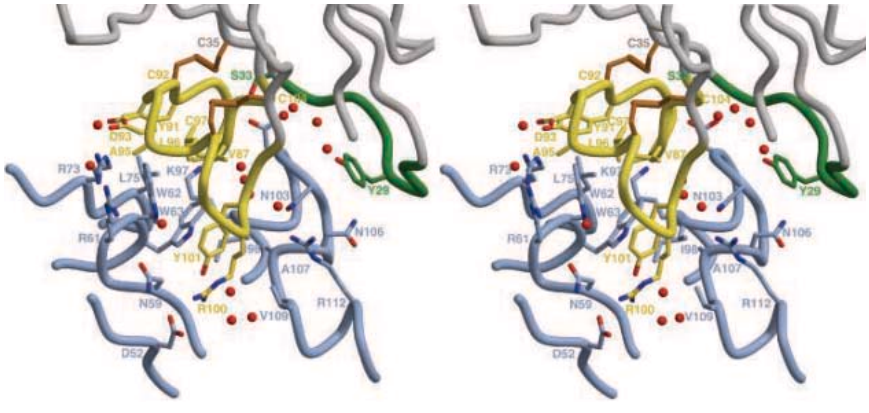

Fig. 4. Stereoview of the IgNAR V region/HEL interface. The main interactions of IgNAR HEL-5A7 (gray) are from CDR3 (yellow) and CDR1 (green) with lysozyme (light blue). Waters are shown as red balls and disulfides in orange. Noninteracting portions of the HEL have been omitted for clarity. The residues with side chains depicted are those that make van der Waals contacts or hydrogen bonds in the complex. A, Ala; C, Cys; D, Asp; I, Ile; K, Lys; L, Leu; N, Asn; R, Arg; S, Ser; V, Val; W, Trp; and Y, Tyr.

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24. Camel and llama V_HH domains share about 65% sequence identity with mouse and human V_H domains; however, several mutations in the canonical V_H interface region render the camelid V_HH domains more soluble than isolated human or murine V_H domains. Additionally, the long CDR3s in several of the camelid V_HH domains are folded back across this interface region, partially rescuing it from solvent exposure. The camelid CDR3 is sometimes anchored to the immunoglobulin core by formation of a disulfide bridge between CDR3 and CDR1 or FR2. Furthermore, some camelid V_HH domains can access recessed cavities, such as the HEL active-site cleft, by means of long CDR3 loops (*12*).
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28. The IgNAR CDR3 is 20 residues in length, spanning N84 to N103 (Gly-Leu-Gly-Val-Ala-Gly-Gly-Tyr-Cys-Asp-Tyr-Ala-Leu-Cys-Ser-Ser-Arg-Tyr-Ala-Glu).
29. Comparison of the IgNAR V domain structure with other antibody x-ray structures with the programs Dali and SSM (*27*) reveals similar root mean square deviations for the IgNAR V domain from V_α, V_L, and V_H domains of around 0.7 to 1.0 Å for 45 core Cα atoms.
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31. The IgNAR CDR1 has root mean square deviations from the cAb-Lys3 and cAb-RN05 CDR1s of 1.2 and 1.5 Å, respectively, for 15 Cα atoms (IgNAR residues N22 to N36).
32. Of the seven residues that differ between HEL and turkey egg-white lysozyme, two ($Arg^{73}\rightarrow Lys^{73}$ and $Asp^{101}\rightarrow Gly^{101}$) are found in the IgNAR-HEL interface. Arg^{L73} makes six van der Waals contacts, one hydrogen bond, and one salt bridge, and Asp^{L101} makes 17 van der Waals contacts and five hydrogen bonds in the interface with IgNAR. The camelid V_HH cAb-Lys3 also binds an epitope encompassing the recessed HEL active site, similarly using its CDR3 for access (*12*). Several murine antibodies to lysozyme (HyHEL10, HyHEL8, HyHEL26, and HyHEL63) recognize a common epitope around the active site, but do not penetrate this site as deeply as do cAb-Lys3 and IgNAR.
33. The camelid anti–ribonuclease A V_HH domain cAb-RN05 uses only CDR1 and CDR3 for binding antigen, whereas the camelid anticarbonic anhydrase V_HH domain cAb-CA05 uses CDR3 almost exclusively with only two van der Waals contacts to the antigen from CDR1. Similarly, the camelid anti–α-amylase V_HH domains AMB7 and AMD10 use all three CDR loops, but CDR1 contributes only 5% of the total buried surface area on the antibody upon antigen binding.
34. The IgNAR-lysozyme interface is comparable in size to those seen in lysozyme-Fab (Fv) complexes that range from 538 to 829 $Å^2$ for the Fab and 540 to 831 $Å^2$ for the lysozyme (table S3). Similar interface sizes have also been observed for lysozyme complexes with camel V_HH domains. A total of 122 van der Waals contacts (table S2), eight hydrogen bonds, and three charged interactions are made between HEL and the IgNAR (table S4). The majority of the buried surface on the IgNAR V domain is contributed by CDR3 residues N85 to N89, N91, N93, N95 to N96, and N98 to N103 (75%), with the remainder by CDR1 N26 to N33 (Fig. 4). The HEL-IgNAR V region interface has a good shape-complementarity index of 0.70 (0.72 and 0.70 for crystal form 2) (*27*), with waters filling several cavities in the interface (fig. S4). A total of 14 water molecules contact both the IgNAR V domain and HEL, with 7 of these (waters 2, 4, 7, 8, 60, 114, and 266) sequestered from contact with external solvent.
35. Type I and type II sequences are 90% identical. IgNAR type II V domains have only four conserved cysteines, rather than the six or more found in type I, and tend toward smaller CDR3 lengths [9 to 18 amino acids (*26*)].
36. The structurally equivalent positions Lys^{N84} (IgNAR) or Lys^{96} (AMD10) correspond to positions Leu^{89} or His^{93} of conventional V domains.
37. IgNAR residues Asn^{N45}, Glu^{N46}, Ser^{N48}, Ser^{N50}, Lys^{N51}, Gly^{N62}, Ser^{N63}, and Lys^{N64} are under strong positive selection.
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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S4
Tables S1 to S4
References and Notes

7 June 2004; accepted 4 August 2004
Published 19 August 2004;
10.1126/science.1101148
Include this information when citing this paper.

Defining a Link with Asthma in Mice Congenitally Deficient in Eosinophils

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Eosinophils are often dominant inflammatory cells present in the lungs of asthma patients. Nonetheless, the role of these leukocytes remains poorly understood. We have created a transgenic line of mice (PHIL) that are specifically devoid of eosinophils, but otherwise have a full complement of hematopoietically derived cells. Allergen challenge of PHIL mice demonstrated that eosinophils were required for pulmonary mucus accumulation and the airway hyperresponsiveness associated with asthma. The development of an eosinophil-less mouse now permits an unambiguous assessment of a number of human diseases that have been linked to this granulocyte, including allergic diseases, parasite infections, and tumorigenesis.

The underlying features of asthma display a marked heterogeneity (*1*, *2*), yet the presence of eosinophils in the airway lumen and lung tissue has been recognized even in the earliest studies (*3*) and is often regarded as a defining feature of this disease (*4*, *5*). Moreover, the recruitment of eosinophils occurs in animal models of allergen-mediated respiratory inflammation; in particular, mouse models have offered unique opportunities with which to examine detailed pathologic features of this disease. However, the availability of clinical studies and numerous mouse models of asthma have not led to an unambiguous description of eosinophil effector functions in asthma, and questions remain as to the specific role(s), if any, of these leukocytes (*6*).

A line of mice devoid of eosinophils was created to test hypotheses that link eosinophils and asthma-related pathogenesis. Transgenic mice devoid of eosinophils were created by lineage-specific expression of a cytocidal protein

with a promoter fragment identified from studies of secondary granule protein genes expressed in mouse eosinophils (*7*–*11*). A candidate promoter from the gene for eosinophil peroxidase (EPO) was selected on the basis of transfection studies with *EPO* promoter–luciferase reporter constructs in the eosinophilic cell line AML14.3D10 (*12*). These studies revealed that upstream sequences from the mouse *EPO* gene were capable of supporting high-level expression that was unique to eosinophil lineage–committed cells (fig. S1). In addition to mouse *EPO*-derived sequences, the transgenic construct developed included the diphtheria toxin A (DTA) chain open reading frame (*13*). The cytocidal character of diphtheria toxin is mediated by the DTA chain (the B chain provides entry into eukaryotic cells) through the catalytic degradation of elongation factor-2 and the subsequent collapse of protein synthesis (*14*).

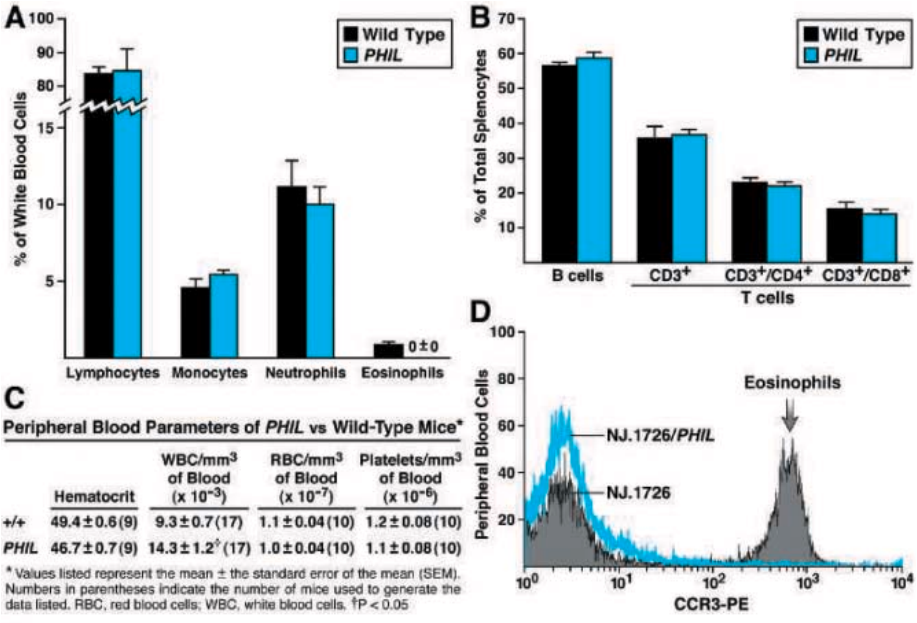

Fig. 1. The eosinophil deficiency of PHIL mice is specific and definitive. (**A**) Peripheral blood of PHIL mice is devoid of eosinophils without effects on the composition of other leukocytes (mean ± SE, $n = 17$ animals per group). (**B**) The targeted loss of eosinophils had no effects on lymphocyte subtypes ($n = 5$ animals per group). (**C**) The specific ablation of eosinophil lineage–committed cells had no additional effects on other hematopoietic parameters, although a nonspecific marginal increase in the steady-state levels of total circulating white blood cells was observed. (**D**) Fluorescence-activated cell sorting analyses demonstrated that the marked blood eosinophilia (i.e., the presence of $CCR3^+$ cells) of the IL-5 transgenic line NJ.1726 (*16*) was completely abolished in NJ.1726/PHIL double transgenic mice. PE, phycoerythrin. (**E**) Immunohistochemistry (dark purple–stained cells) with eosinophil-specific rabbit polyclonal antisera to MBP demonstrates that tissues or organs with prominent resident populations of eosinophils at baseline in wild-type mice were devoid of these granulocytes in PHIL mice. Scale bar, 100 μm.

Assessment of circulating leukocytes (*15*) in the resulting *EPO-DTA* transgenic (PHIL) mice demonstrated that these animals were devoid of eosinophils but otherwise have a full complement of hematopoietically derived cells (Fig. 1A). An examination of splenic lymphoid cells (*15*) revealed normal numbers of B cells, T cells, and the T lymphocyte $CD4^+$ and $CD8^+$ subtypes (Fig. 1B). Assessments of lung sections and peritoneal cavity exudates from PHIL mice revealed wild-type levels of mast cells (fig. S2, A and B). Moreover, circulating basophils were identified in peripheral blood from PHIL mice (fig. S2C), demonstrating that even a leukocyte lineage sharing a direct common precursor with the eosinophil lineage was unaffected. The specific ablation of eosinophils in PHIL mice also occurred with no effects on either erythropoiesis or the production of platelets (Fig. 1C). A nominal elevation of total white blood cell counts was consistently observed in PHIL mice relative to negative littermates. This increase, however, was not specific to any one cell type and did not elevate circulating cell numbers beyond the normal observable range in wild-type mice.

The loss of eosinophils in PHIL mice was nearly absolute, with only an occasional eosinophil identified in surveys of blood films from 1 of 20 animals examined. This eosinophil deficiency is lifelong and a Mendelian inheritable trait of the line. The specificity of the eosinophil deficiency in PHIL mice was achieved through a cross with interleukin (IL)–5 transgenic animals (*16*). These *IL-5* transgenic mice have circulating eosinophil levels that, in some cases, exceed 100,000 per mm^3 of blood, representing ~50% of all white blood cells. Analyses of blood from double transgenic animals (i.e., mice carrying both the *DTA* and *IL-5* transgenes) again revealed a complete absence of eosinophils (Fig. 1D).

The eosinophil-deficient character of PHIL mice was extended further by immunohistochemistry with antibodies specific for Major Basic Protein (MBP) (*15*, *17*, *18*). Tissues with abundant resident populations of eosinophils (i.e., bone marrow, uterus, small

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intestines, and thymus) in wild-type animals were shown to be devoid of these granulocytes in PHIL mice (Fig. 1E).

PHIL mice were subjected to an acute allergen sensitization/aerosol challenge model of asthma (*15*) to determine if the presence of eosinophils was causatively linked to the development of disease symptoms. Whereas wild-type mice sensitized/aerosol challenged with chicken ovalbumin (OVA) developed a significant airway eosinophilia [~50% of bronchoalveolar lavage fluid (BAL) cells], PHIL mice were essentially devoid of eosinophils, with only trace numbers (<0.5%) of eosinophils identifiable in the BAL of one of four OVA-treated animals (Fig. 2A). The loss of eosinophils from the lungs of OVA-treated PHIL mice also extended to tissue-infiltrating cells. Specifically, the lungs of OVA-treated PHIL mice were devoid of eosinophils, unlike the significant tissue eosinophilia that occurred in the areas surrounding the central airways (peribronchial) and the vasculature (perivascular) of OVA-treated wild-type animals (Fig. 2, B to D, and fig. S3). Examination of blood films and bone marrow smears from OVA-treated PHIL animals again revealed only an occasional eosinophil in a fraction of the animals examined.

The targeted ablation of eosinophils had significant effects on allergen-induced pulmonary pathology, suggesting a causative role for these granulocytes. Overall, OVA-induced histopathology in PHIL mice was attenuated relative to OVA-treated wild-type littermates. This lack of pathology was manifested by the reduced airway epithelial hypertrophy in OVA-treated PHIL mice (Fig. 2, B and C). In addition, assessment of airway mucins by periodic acid–Schiff (PAS) staining (*15*) demonstrated that OVA-induced goblet cell metaplasia/mucus accumulation (GM/MA) in PHIL mice was significantly reduced (Fig. 3, A to C). A quantitative assessment of the staining in these tissue sections revealed a 68% reduction of PAS staining in PHIL mice relative to OVA-treated wild-type animals (Fig. 3D). However, the GM/MA observed in OVA-treated PHIL mice was still significant when compared to allergen-naive animals. This observation suggests that eosinophils contribute to, and are necessary for, the levels of pathology observed in wild-type mice, but that they are not alone sufficient to account for these wild-type levels. That is, both eosinophil-dependent and -independent mechanisms exist in the lung that elicit GM/MA after allergen challenge.

The association between allergen-induced pulmonary eosinophilia and the development of lung dysfunction in both asthma patients and mouse models has been, at best, a collection of confusing and often contradictory observations. The lack of symptom improvement in asthma patients after administration of antibodies to IL-5 exemplifies the ambiguous character of clinical studies that attempt to ablate eosinophils (*6*). Mouse models purporting to ablate eosinophils

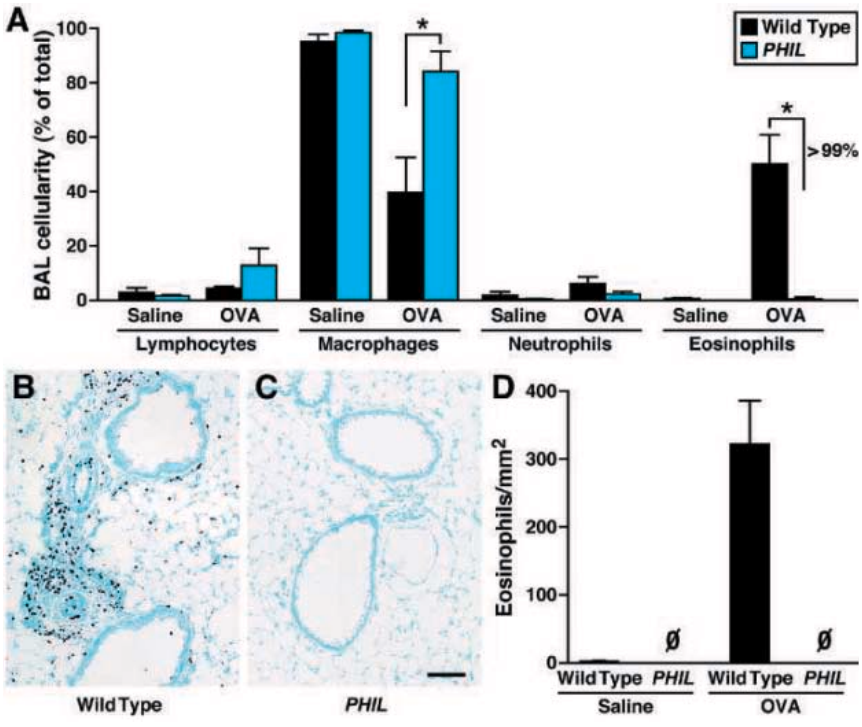

Fig. 2. The pulmonary eosinophilia associated with OVA sensitization/aerosol challenge was abolished in PHIL mice. (**A**) OVA-induced eosinophilia of the airway lumen was lost (decreased by >99%) in PHIL mice (mean ± SE, $n = 5$ animals per group). *, $P < 0.001$. (**B** and **C**) Assessments of infiltrating eosinophils in (B) wild-type and (C) PHIL mice by immunohistochemistry (dark purple–stained cells) with rabbit polyclonal antisera to mouse MBP revealed that OVA-induced accumulation of eosinophils was also extinguished in PHIL mice. Scale bar, 100 μm. (**D**) Quantitative assessments of the number of eosinophils infiltrating peribronchial areas (i.e., eosinophils per mm^2) demonstrated that OVA-treated PHIL mice were devoid of tissue eosinophils (mean ± SE, $n = 5$ animals per group). Ø indicates the absence of eosinophils in any of the sections of any of the mice in the cohort examined.

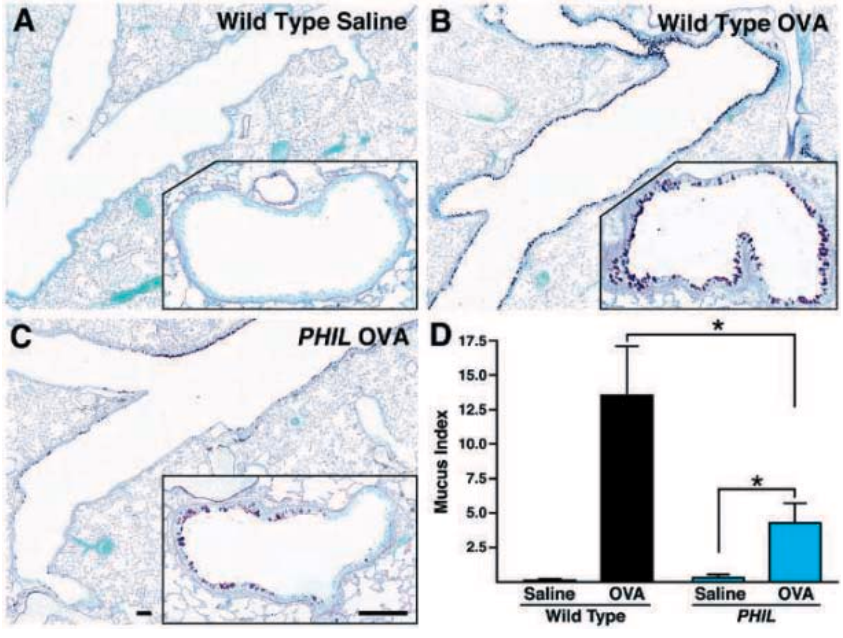

Fig. 3. The specific loss of eosinophils in PHIL mice resulted in a significant reduction in OVA-induced GM/MA. Representative lung sections after PAS staining are shown for (**A**) saline control and (**B**) OVA sensitized/OVA aerosol challenged wild-type mice in comparison to (**C**) OVA sensitized/OVA aerosol challenged PHIL mice. The sections of each panel show early-branching central conducting airways, whereas the insets show smaller, more distal bronchioles. Scale bars, 100 μm. (**D**) Quantitative assessments of airway epithelial mucus content showed a marked decrease (relative to wild type) in PHIL mice (mean ± SE, 5 to 10 animals per group). All evaluations of histopathology were performed in duplicate as independent observer-blinded assessments. *, $P < 0.05$.

are also ambiguous, as they either do not completely eliminate pulmonary eosinophils or they elicit the loss of eosinophils by mechanisms that do not differentiate between effects on eosinophils and other potentially important cellular targets (*19*–*22*). However, measurements of lung function after OVA sensitization/aerosol challenge of PHIL mice (*15*) showed that methacholine-induced airway hyperresponsiveness was dependent on the presence of eosinophils (Fig. 4). Moreover, the specific loss of eosinophils also led to improvement of other pulmonary function parameters associated with the distal regions of the lung (fig. S4).

The lack of observable phenotypes in knockout mice deficient for the abundant secondary granule proteins MBP-1 (*18*) and EPO (*17*) suggests that activities other than degranulation, including antigen presentation (*23*), the release of small molecule mediators of inflammation [e.g., the synthesis and release of eicosanoid mediators of inflammation (*24*)], and immune regulation of the pulmonary microenvironment through either modulations of T cell activities (*21*) or eosinophil-derived cytokine and/or chemokine expression (*25*) are likely to be the relevant effector functions. Eosinophil-derived cytokine and/or chemokine expression, in particular, is noteworthy as it may account for the chronic and seemingly self-sustaining character of allergic pulmonary inflammation, which often leads to lung remodeling events (*26*, *27*). Significant decreases of Th2 cytokine levels in BAL of OVA-treated PHIL mice (*28*) lend support to this hypothesis and suggest that a prominent eosinophil effector function in the lung is localized immune regulation.

This study shows that eosinophil activities are important contributory factors leading to symptoms that are classically defined as hallmark features of asthma. More importantly, these data provide validation of earlier studies that independently concluded that a causative link exists between eosinophils and allergic pulmonary pathologies (*22*, *29*). The dependency of allergen-induced pulmonary pathologies on eosinophils suggests that these granulocytes participate at a significant level in underlying inflammatory responses. Regardless of the ultimate definition of the causative activities mediated by eosinophils, the challenge of future studies will be to develop confirmatory clinical studies to unambiguously define the role(s) and extent of eosinophil effector functions in asthma patients. The results of such studies will not only widen our understanding of the principle causes of asthma, but are also likely to lead to targeted therapeutic approaches previously dismissed and/or overlooked.

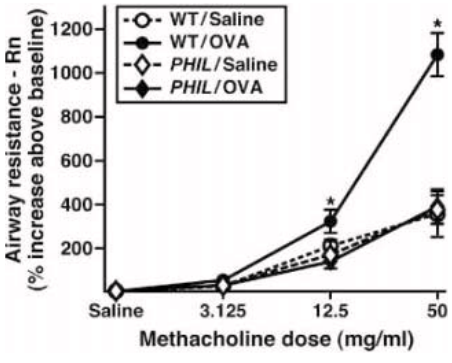

Fig. 4. In the absence of eosinophils, OVA-induced airway hyperresponsiveness does not develop. Lung function was assessed as airway resistance (Rn) in response to aerosolized methacholine, in saline-treated (WT/Saline) and OVA sensitized/OVA aerosol challenged (WT/OVA) wild-type mice in comparison to saline-treated (PHIL/Saline) and OVA sensitized/OVA aerosol challenged (PHIL/OVA) PHIL mice (n = 5 to 10 animals per group). Asterisks indicate a significant difference ($P < 0.01$) between WT/OVA and either WT/Saline, PHIL/Saline, or PHIL/OVA mice.

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30. We wish to acknowledge the kind gift of the gene encoding DTA by I. Maxwell. We also thank the Mayo Clinic Arizona Core Facilities (L. Barbarisi, T. Brehm-Gibson, S. Savarirayan, M. Ruona, B. Schimeck, J. Caplette, and J. Protheroe) and our administrative staff (L. Mardel, J. Ford, and P. McGarry). Supported by the Mayo Foundation; an American Heart Association grant to J.J.L. (no. 045580Z); grants from NIH to J.J.L. (no. HL065228), N.A.L. (no. HL058723), S.J.A. (nos. AI033043 and AI025230), and C.G.I. (nos. NCRR-COBRE P20RR15557, P01-HL67004, and HL-EB67273); and by NIH postdoctoral fellowships to MM.P.M. (no. HL10105) and S.A.C. (no. AR08545).

Supporting Online Material

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22 April 2004; accepted 23 July 2004

A Critical Role for Eosinophils in Allergic Airways Remodeling

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Features of chronic asthma include airway hyperresponsiveness, inflammatory infiltrates, and structural changes in the airways, termed remodeling. The contribution of eosinophils, cells associated with asthma and allergy, remains to be established. We show that in mice with a total ablation of the eosinophil lineage, increases in airway hyperresponsiveness and mucus secretion were similar to those observed in wild-type mice, but eosinophil-deficient mice were significantly protected from peribronchiolar collagen deposition and increases in airway smooth muscle. These data suggest that eosinophils contribute substantially to airway remodeling but are not obligatory for allergen-induced lung dysfunction, and support an important role for eosinophil-targeted therapies in chronic asthma.

Since its discovery by Paul Erlich in 1879, there has been a wealth of information documenting the association between eosinophils and parasitic or allergic diseases (*1*). The role of eosinophils in allergic disease remains controversial. Although T helper cell 2 (T_H2) lymphocytes are thought to drive asthmatic responses, increasing evidence suggests that eosinophils are associated with development of lung dysfunction and subsequent immunopathology (*2*–*4*).

Asthma is a chronic disease characterized by airway hyperresponsiveness (AHR), airway inflammation, and reversible airway ob-

struction. In addition, structural changes in the airway, termed remodeling, occur as a result of an imbalance in tissue regeneration and repair mechanisms (*5*, *6*). Subepithelial fibrosis is a distinctive feature of airway remodeling and contributes to the thickened airway walls due to the deposition of collagen types I, III, and IV, fibronectin, and other extracellular matrix (ECM) proteins such as tenascin and laminin (*7*, *8*). Increased airway smooth muscle (ASM) mass and excessive mucus secretion from hyperplastic goblet cells are also features of airway remodeling (*9*, *10*).

To define the role of eosinophils in asthma pathophysiology, we used the recently described eosinophil lineage–ablated line, ΔdblGATA mice (*11*). Deletion of a high-affinity GATA site in the GATA-1 promotor results in a complete ablation of the eosinophil lineage without affecting the development of the other GATA-1–dependent lineages (erythroid, megakaryocytic, and mast cell) (*11*).

We examined the extent of this mutation on eosinophil recruitment following acute and chronic allergen challenge in a murine model of allergic airways disease (*12*, *13*). Histological examination confirmed that sham-treated ΔdblGATA mice were completely devoid of eosinophils and that allergen challenge failed to induce eosinophilia in the airways and bone marrow of ΔdblGATA mice (Fig. 1, A to D). Eosinophil peroxidase (EPO) analysis (*13*) of lung tissue (fig S2) and bone marrow (Fig. 1E) confirmed the absence of eosinophils in these tissues. During acute and chronic phases, wild-type (WT) mice showed significant increases in pulmonary eosinophils and lymphocytes. Allergen challenge induced similar numbers of alveolar macrophages and lymphocytes in both WT and ΔdblGATA mice, confirming that the ΔdblGATA mutation was selective for eosinophils [bronchoalveolar lavage (BAL) cell counts and lung EPO are shown in figs. S1 and S2)]. Given the role of GATA-1 in mast cell differentiation (*14*), histological analysis of chloroacetate esterase–stained tissue sections demonstrated that this mutation had no effect on mast cell numbers (*15*).

Airway function was assessed using whole-body plethysmography (*13*). Pulmonary conductance (GL) and compliance (Cdyn) (*16*), and Penh [a calculated value that correlates with measurement of airway resistance, obstruction, and intrapleural pressure in the same mouse (*17*)] were assessed on day 25 after acute challenge on days 21 to 24. WT allergen-challenged mice developed a significantly enhanced response to methacholine (Mch) when compared to WT sham-treated animals. In the absence of eosinophils, allergen-challenged ΔdblGATA mice displayed enhanced responses to Mch relative to baseline sham controls that were comparable to those displayed by challenged WT mice (Fig. 2, A and B). Similarly, the Penh responses of ovalbumin (OA)-challenged ΔdblGATA mice to Mch were almost identical to those of their WT-challenged counterparts (fig. S3). During the chronic phase, mice were assessed for changes in lung function weekly until day 55. Although the enhancement following allergen challenge was lower than that seen during the acute phase (at day 25), allergen-challenged WT and ΔdblGATA mice displayed similar enhanced responses to cholinergic stimulation relative to baseline sham controls (fig. S4). Thus, eosinophil deficiency conferred no protection against Mch-induced AHR (during acute and chronic allergen challenge), suggesting that eosinophils are not obligatory for allergen-induced changes in airway physiology.

Given the role of T_H2 cells in the allergic response, we examined T_H2 cytokine expression in the lungs of acute and chronically challenged WT and ΔdblGATA mice. T_H2 responses in ΔdblGATA mice appeared normal and were similar to those of their WT littermates. ΔdblGATA mice displayed increased BAL and lung interleukin-4 (IL-4),

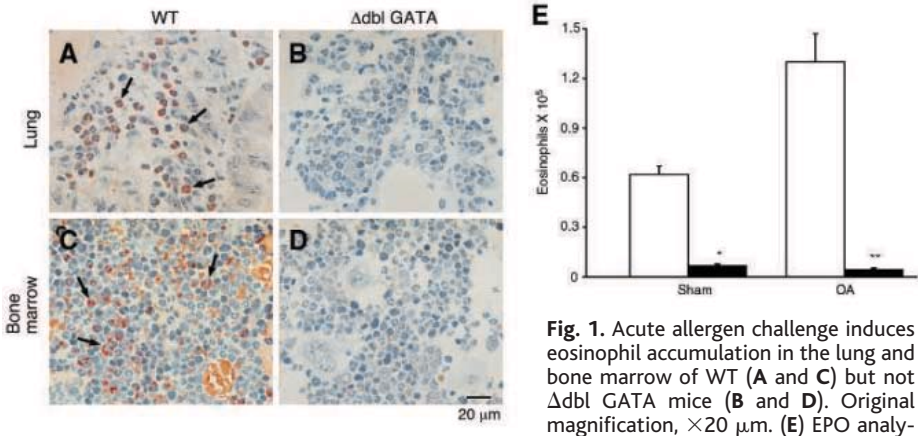

Fig. 1. Acute allergen challenge induces eosinophil accumulation in the lung and bone marrow of WT (**A** and **C**) but not ΔdblGATA mice (**B** and **D**). Original magnification, ×20 μm. (**E**) EPO analysis of bone marrow confirmed that ΔdblGATA mice (solid bars) are devoid of eosinophils compared to WT controls (open bars) before (sham) and after (OA) allergen challenge. Results are means ± SEM (Sham, $n = 4$ mice; OA, $n = 5$ mice). Significant differences between respective sham-treated and sensitized/challenged WT or ΔdblGATA mice are indicated as $*P < 0.03$ and $**P < 0.008$.

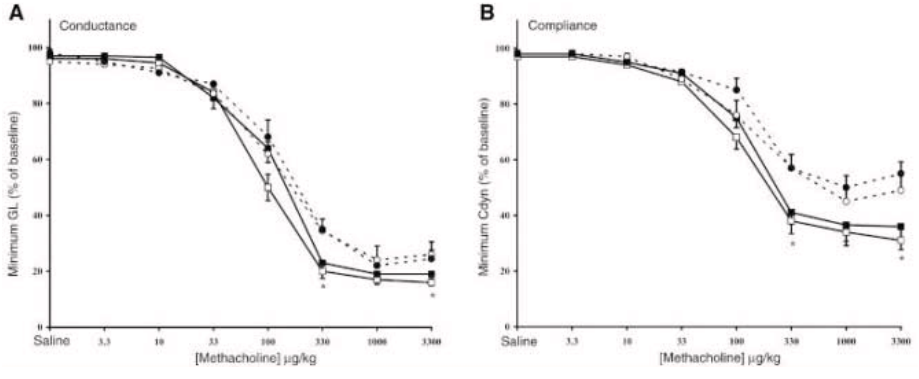

Fig. 2. AHR following acute allergen challenge. Sham-treated (dashed lines) WT (○) or ΔdblGATA mice (●) mice were exposed to aerosolized saline, and OA-sensitized (solid lines) WT (□) and ΔdblGATA mice (■) were exposed to aerosolized OA on days 21 to 24. About 21 to 24 hours after the last aerosol challenge, mice were anesthetized, intubated, and mechanically ventilated, and airway responses to increasing concentrations of intravenous Mch were assessed. The dose-response curves for (**A**) pulmonary conductance (GL) and (**B**) pulmonary compliance (Cdyn) are shown. Results are means ± SEM (Sham, $n = 4$ mice; OA, $n = 8$ or 9 mice) of the percentage minimal decrease in pulmonary conductance or compliance obtained after Mch challenge compared with the baseline value just before challenge. Significant differences between respective sham-treated and sensitized/challenged WT or ΔdblGATA mice are indicated as $*P < 0.05$ to $P < 0.01$.

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IL-5, and IL-13 protein following acute allergen challenge. Likewise, IL-4 and IL-5 expression during the chronic phase were comparable for WT and Δdbl GATA mice (fig. S5). Numbers of T_H2 cells, determined by staining lungs for the T_H2 surface marker T1/ST2, were found to be comparable between WT and Δdbl GATA mice (*15*). Moreover, serum-specific OA–immunoglobulin E was similar (acute OA WT = 3713 ± 539 ng/ml versus OA Δdbl GATA mice = 4059 ± 789 ng/ml; chronic OA WT = 5204 ± 716 ng/ml versus OA Δdbl GATA mice = 5635 ± 741 ng/ml; n = 6 to 9 mice). These data demonstrate that allergen-driven T_H2 responses develop in the absence of eosinophils.

T_H2 cytokines (IL-4, IL-5, IL-9, and IL-13) and transforming growth factor–β (TGF-β) have been shown to induce subepithelial fibrosis (*18*–*24*). Recent reports support a potential role for eosinophils in the development of airway remodeling (*2*–*4*). Increased eosinophils in the bronchial mucosa of severe asthmatics have been associated with basement membrane thickening (*25*), and eosinophils are capable of secreting an array of profibrotic mediators (*22*, *23*). However, studies in which IL-5 activity was inhibited, although associated with a decrease in eosinophil numbers, could theoretically be operating on a number of pathways independent of the eosinophil (*2*–*4*). In light of this ambiguity, we examined the effects of specific eosinophil deficiency on airway remodeling following chronic challenge.

Increased mucus secretion from hyperplastic goblet cells, shown by periodic acid-Schiff (PAS)–positive cells in the bronchial epithelium (*13*), was similar in WT and Δdbl GATA mice compared to sham controls after acute challenge, and these increases were sustained throughout chronic challenge (fig. S6). Thus, enhanced mucus secretion occurs in allergic airways independent of eosinophils.

Increased subepithelial deposition of ECM proteins, specifically collagen, is a prominent feature of airway remodeling. We examined matrix deposition (collagen and fibrin) in lung sections stained with Martius scarlet blue (MSB) (*13*, *26*). Sham mice showed a thin uniform layer of matrix in peribronchiolar subepithelial regions (Fig. 3, A and B), whereas acute challenge marginally increased fine matrix in both WT and Δdbl GATA mice within some infiltrates (*15*). Prolonged challenge of WT mice significantly increased matrix deposition in the subepithelial layer of the bronchioles and perivascular regions. Dense fibrils were seen in the subepithelial and submucosal areas and in between the inflammatory cells. In marked contrast, matrix deposition in these same regions was consistently reduced in Δdbl GATA mice when compared with that in WT mice (Fig. 3, A to D; fig. S7, A to D). Quantitative image analysis of MSB-stained lung sections and biochemical measurement of total collagen in lung tissue (*13*) confirmed that prolonged allergen challenge of WT mice provoked a marked increase (up to threefold) in matrix deposition, as compared with that seen in sham mice, and levels were significantly reduced in challenged Δdbl GATA mice (Fig. 3, E and F, respectively). These results conclusively demonstrate that eosinophils contribute to allergen-induced subepithelial collagen deposition.

The effects of eosinophil deficiency on ASM hyperplasia and proliferation were determined by counting the numbers of total and proliferating cell nuclear antigen (PCNA)–positive smooth muscle cells along the basement membrane of three or four bronchioles per animal (*13*). Prolonged allergen challenge of WT mice induced a significant increase in the total and proliferating number of ASM cells compared with that seen in sham mice. This increase was absent from airways of chronically challenged Δdbl GATA mice (Fig. 4 and fig. S8). Prolonged allergen challenge induces phenotypic changes in ASM cells (*27*), which could conceivably induce secretion of a number of growth factors, like TGF-β, which contribute to ECM formation. We investigated expression of active TGF-β1 in WT and Δdbl GATA mice and consistently found no differences in TGF-β1 expression between chronically challenged WT and Δdbl GATA mice (either protein or mRNA). These data suggest that reduced subepithelial fibrosis in our model is independent of TGF-β1 expression.

Our work contrasts with a recent report demonstrating that IL-5–deficient mice are protected from collagen deposition because of a reduction in TGF-β–positive eosinophils (*4*). We have previously shown that mononuclear cells, presumably macrophages, and not

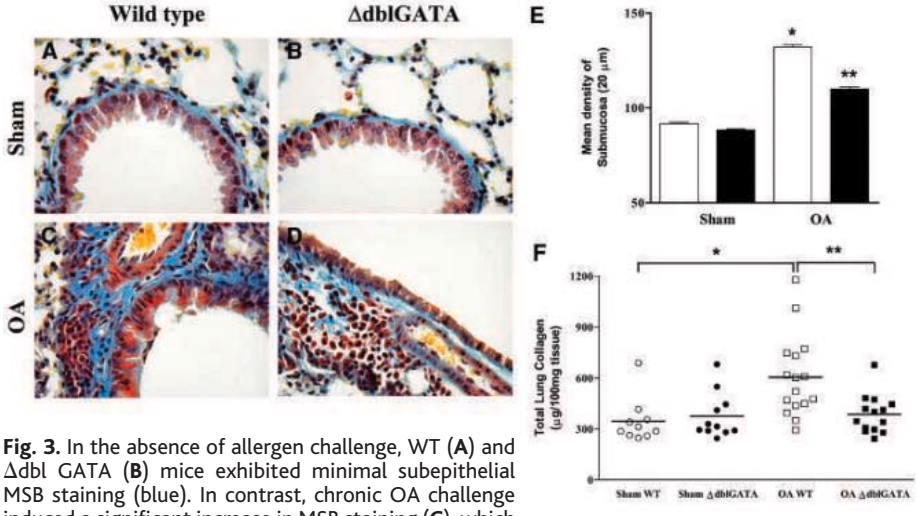

Fig. 3. In the absence of allergen challenge, WT (**A**) and Δdbl GATA (**B**) mice exhibited minimal subepithelial MSB staining (blue). In contrast, chronic OA challenge induced a significant increase in MSB staining (**C**), which was markedly reduced in challenged Δdbl GATA mice (**D**). Data are representative of 8 to 12 mice per group; original magnifications, ×40. (**E**) Image analysis of MSB-stained lung sections from sham or chronically challenged WT (open bars) and Δdbl GATA mice (solid bars) confirmed that challenged Δdbl GATA mice were significantly protected from collagen deposition (**$P < 0.0001$). Results are means ± SEM (n = 8 to 12 mice per group). Significance between Sham WT and OA WT is indicated (*$P < 0.0001$). (**F**) Lung collagen was measured in sham and chronically challenged Δdbl GATA mice. Individual values and means (solid lines) for each group are shown (n = 10 to 16 mice per group). Significant differences between Sham WT and OA WT, and between OA WT and Δdbl GATA mice, are indicated as *$P < 0.001$ and **$P < 0.003$, respectively.

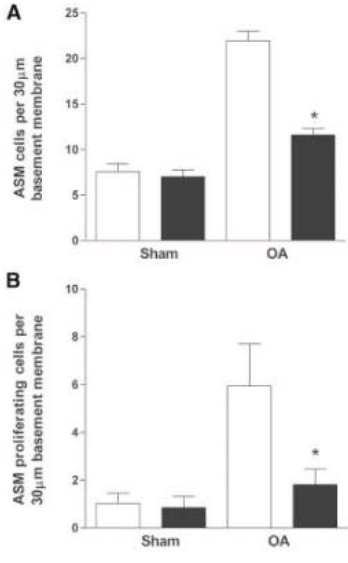

Fig. 4. Eosinophil deficiency protects against increases in ASM. (**A**) Number of ASM cells (round and elongated) and (**B**) proliferating (PCNA-positive) ASM cells along the basement membrane of three or four bronchioles per mouse were determined in sham and chronically challenged WT (open bars) and Δdbl GATA mice (solid bars). Results are means ± SEM for each group (Sham, n = 4 mice; OA, n = 6 mice). Significant difference between OA WT and Δdbl GATA mice is indicated (*$P < 0.004$).

eosinophils are the main secretors of TGF-β1 protein during chronic challenge (*12*). The reason for this disparity is unclear (*4*), but variability in protocols may account for the differences seen in the cell source and expression levels of TGF-β. A number of other factors have been demonstrated to be profibrotic in the lung, notably the chemokine MCP-1, thrombin, endothelin-1, and plasminogen activator inhibitor 1 (*28*). It is difficult to link the presence of these factors to eosinophils specifically. However, the cysteinyl leukotrienes have been shown to be linked to both profibrotic remodeling responses and eosinophils (*29*, *30*). In fact, the eosinophil may be a major source of leukotrienes, often overlooked.

Of importance is that these animal studies are in accordance with observations made in humans. Mild asthmatic patients pretreated with IL-5–specific antibody exhibited significant reduction in tenascin, lumican, and procollagen III (*3*). Our results independently demonstrate that eosinophils are in part responsible for both collagen and smooth muscle changes in a chronic model of asthma. Further, although the contribution of eosinophils to lung dysfunction has been controversial, we show here that eosinophils are not obligatory for airway physiology changes associated with this disease. Taken together, these data provide a rationale for anti-eosinophil–based therapeutics in chronic allergic airways disease.

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31. We thank J. Brewer for technical assistance and the staff of Animal Resources Children's Hospital for animal care. This work was supported by the NIH (grants AI39759 and HL10463). C.M.L. and S.J.M. were supported by the Wellcome Trust (award 057704) and G.X. by the European Molecular Biology Foundation.

Supporting Online Material

www.sciencemag.org/cgi/content/full/305/5691/1776/DC1
Materials and Methods
Figs. S1 to S8
References

13 May 2004; accepted 21 July 2004

Children Creating Core Properties of Language: Evidence from an Emerging Sign Language in Nicaragua

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A new sign language has been created by deaf Nicaraguans over the past 25 years, providing an opportunity to observe the inception of universal hallmarks of language. We found that in their initial creation of the language, children analyzed complex events into basic elements and sequenced these elements into hierarchically structured expressions according to principles not observed in gestures accompanying speech in the surrounding language. Successive cohorts of learners extended this procedure, transforming Nicaraguan signing from its early gestural form into a linguistic system. We propose that this early segmentation and recombination reflect mechanisms with which children learn, and thereby perpetuate, language. Thus, children naturally possess learning abilities capable of giving language its fundamental structure.

Certain properties of language are so central to the way languages operate, and so widely observed, that Hockett termed them "design features" of language (*1*). This study asks whether these properties can arise naturally as a product of language-learning mechanisms, even when they are not available in the surrounding language environment. We focus here on two particular properties of language: discreteness and combinatorial patterning. Every language consists of a finite set of recombinable parts. These basic elements are perceived categorically, not continuously, and are organized in a principled, hierarchical fashion. For example, we have discrete sounds that are combined to form words, that are combined to form phrases, and then sentences, and so on. Even those aspects of the world that are experienced as continuous and holistic are represented with language that is discrete and combinatorial. Together, these properties make it possible to generate an infinite number of expressions with a finite system. It is generally agreed that they are universal hallmarks of language, although their origin is the subject of continued controversy (*2*–*7*).

Humans are capable of representations that lack these properties. For example, nonlinguistic representations such as maps and paintings derive their structure iconically, from their referent. That is, patterns in the representation correspond, part for part, to patterns in the thing represented. In this way, half a city map represents half a city. Unlike language, such nonlinguistic representations are typically analog and holistic.

The present study documents the emergence of discreteness and combinatorial patterning in a new language. Over the past 25 years, a sign language has arisen within a community of deaf Nicaraguans who lacked exposure to a developed language. This situation enables us to discover how fundamental language properties emerge as the nonlinguistic becomes linguistic.

Before the 1970s, deaf Nicaraguan children and adults had little contact with each other. Societal attitudes kept most deaf individuals at home, and the few schools and clinics available

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served small numbers of children. Interviews with former students reveal little evidence of contact with classmates outside school, or after graduation (*8*, *9*). In this context, no sign language emerged, as evidenced by the lack of language in today's adults over the age of 45.

In such situations, deaf people will often develop "home signs": communication systems built up out of common gestures, used with family members. Although not full languages, home signs exhibit some of the rudiments of language (*10*, *11*). The home sign systems developed by Nicaraguans appear to have varied widely from one deaf person to another in form and complexity (*12*).

This situation changed abruptly with the opening of an expanded elementary school for special education in 1977, followed by a vocational school in 1981, both in Managua. Deaf enrollment in the programs initially comprised about 50 students, growing to more than 200 by 1981 and increasing gradually throughout the 1980s. For the first time, students continued their contact outside school hours, and by the mid-1980s deaf adolescents were meeting regularly on the weekends (*8*). Although instruction in school was conducted in Spanish (with minimal success), these first children began to develop a new, gestural system for communicating with each other. The gestures soon expanded to form an early sign language (*13*, *14*). Through continued use, both in and out of school, the growing language has been passed down and relearned naturally every year since, as each new wave of children entered the community (*15*).

Today there are about 800 deaf signers of Nicaraguan Sign Language (NSL), ranging from 4 to 45 years of age. Previous research on NSL has found that changes in its grammar first appear among preadolescent signers, soon spreading to subsequent, younger learners, but not to adults (*16*). This pattern of transmission, when combined with the rapid and recent expansion of NSL, has created an unusual language community in which the most fluent signers are the youngest, most recent learners. Consequently, much of the history of the language can be surveyed by performing a series of observations, progressing from the older signers, who retain much of NSL's early nature, to younger, more recent learners, who produce the language in its expanded, most developed form.

Following this logic, the present study compares the signed expressions of 30 deaf Nicaraguans, grouped into cohorts according to the year that they were first exposed to NSL: 10 from a first cohort (before 1984), 10 from a second cohort (1984 to 1993), and 10 from a third cohort (after 1993). All of the deaf participants have been signing NSL since the age of 6 or younger. Their signed expressions are compared to the gestures produced by 10 hearing Nicaraguan Spanish speakers while speaking Spanish (*17*).

In particular, we examine the gestures and signs in expressions that describe complex motion events, such as rolling down a hill or climbing up a wall. We chose descriptions of motion for two reasons. First, previous research has found that when speakers describe motion events, they often produce co-speech gestures that iconically represent the movement (*18*, *19*). Such gestures (unlike speech) are fully available to deaf observers, likely providing raw materials to shape into a sign language. Second, the description of motion offers a promising domain for detecting the introduction of segmented, linear, and hierarchical organization of information into a communication system. Motion events include a manner of movement (such as rolling) and a path of movement (such as descending). These characteristics of motion are simultaneous aspects of a single event and are experienced holistically. The most direct way to iconically represent such an event would be to represent manner and path simultaneously. Languages, in contrast, typically encode manner and path in separate elements, combined according to the rules of the particular language (*20*). For example, English produces one word to express manner (rolling) and another to express path (down), and assembles them into the sequence "rolling down." Signing that dissects motion events into separate manner and path elements, and assembles them into a sequence, would exhibit the segmentation and linearization typical of developed languages and unlike the experience of motion itself.

To collect samples of signing and gesturing that describe motion events, we presented participants with an animated cartoon and videotaped them telling its story to a peer. Deaf subjects signed their narratives. Hearing subjects spoke Spanish, and only their co-speech gestures were analyzed. Those expressions that included both manner and path information were coded with respect to how the information was integrated: (i) simultaneously, as a single hand movement, and/or (ii) sequentially, articulated separately within a string of simple manner-only and path-only elements (Fig. 1). Note that a single multigesture expression can include both means of integration.

Two analyses compared, across groups, the use of each method of integration. Figure 2A shows the proportion of the expressions produced by each participant that include manner and path simultaneously. All of the Spanish speakers' gestures (1.0) and most of the first-cohort signers' expressions (0.73) use this approach. Second- and third-cohort signers produce relatively fewer expressions of this type (0.32 and 0.38). Figure 2B shows the proportion of expressions produced by each participant that articulate manner and path sequentially. Such sequences are never observed in the Spanish speakers' gestures (0). First-cohort signers sometimes include such sequences (0.27); second- and third-cohort signers include such sequences in most of their expressions (0.78 and 0.73).

In appearance, the signs very much resembled the gestures that accompany speech.

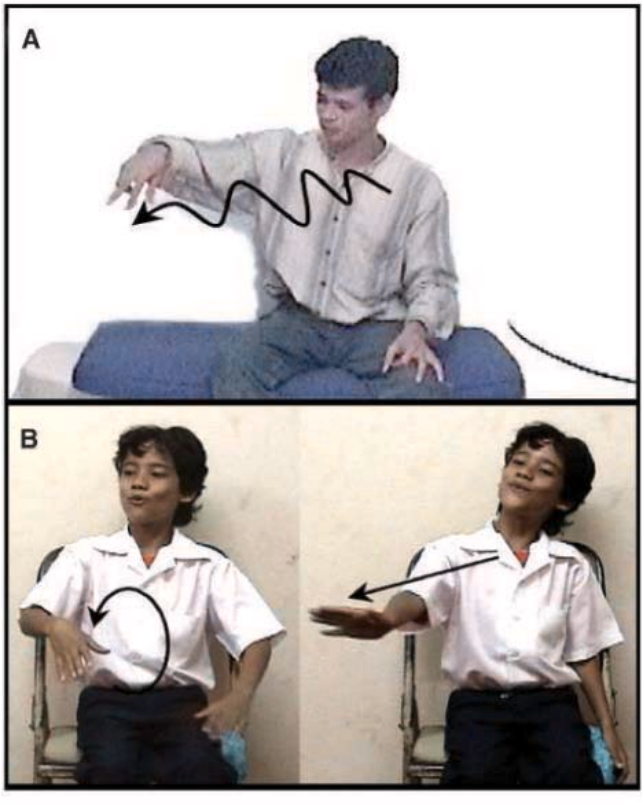

Fig. 1. Examples of motion event expressions from participants' narratives. (**A**) Manner and path expressed simultaneously. This example shows a Spanish speaker describing an event in which a cat, having swallowed a bowling ball, proceeds rapidly down a steep street in a wobbling, rolling manner. The gesture shown here naturally accompanies his speech. Here, manner (wiggling) and path (trajectory to the speaker's right) are expressed together in a single holistic movement. (**B**) Manner and path expressed sequentially. This example shows a third-cohort signer describing the same rolling event in Nicaraguan Sign Language. Here, manner (circling) and path (trajectory to signer's right) are expressed in two separate signs, assembled into a sequence. (The video clips from which the frames were drawn can be viewed at *Science* Online.)

The movements of the hands and body in the sign language are clearly derived from a gestural source. Nonetheless, the analyses reveal a qualitative difference between gesturing and signing. In gesture, manner and path were integrated by expressing them simultaneously and holistically, the way they occur in the motion itself. Despite this analog, holistic nature of the gesturing that surrounded them, the first cohort of children, who started building NSL in the late 1970s, evidently introduced the possibility of dissecting out manner and path and assembling them into a sequence of elemental units. As second and third cohorts learned the language in the mid-1980s and 1990s, they rapidly made this segmented, sequenced construction the preferred means of expressing motion events. NSL thus quickly acquired the discrete, combinatorial nature that is a hallmark of language.

Note that this change to the language, in the short term, entails a loss of information. When representations express manner and path separately, it is no longer iconically clear that the two aspects of movement occurred simultaneously, within a single event. For example, *roll* followed by *downward* might have instead referred to two separate events, meaning "rolling, then descending."

However, the communicative power gained by combining elements more than offsets this potential for ambiguity. Elements and sequencing provide the building blocks for linguistic constructions (such as phrases and sentences) whose structure assigns meaning beyond the simple sum of the individual words. We observed one such structured sequence pattern that has emerged specifically for expressing simultaneity. A sign can be produced before and after another sign or phrase in an A-B-A construction, essentially embedding the second element within the first, yielding expressions such as *roll descend roll*. This string can serve as a structural unit within a larger expression like *cat [roll descend roll]*, or can even be embedded within another sign, as in *waddle [roll descend roll] waddle*, and so on. These A-B-A constructions appeared in about one-third of the coded expressions (0.37) by participants from all three cohorts: four first-cohort signers, seven second-cohort signers, and six third-cohort signers. They were used to link various simultaneous aspects of events, including agent and action (*cat climb cat*), ground and action (*climb pipe climb*), and manner and path (*roll descend roll*). We observed 15 examples of these constructions applied specifically for combining manner and path information, again by signers of all three cohorts: two first-cohort signers, four second-cohort signers, and four third-cohort signers. They never appeared in the gestures of the Spanish speakers, and they represent a temporal hierarchy not found in motion events themselves.

Such hierarchical combinations are central to the language engine, enabling the production of an infinite set of utterances with a finite set of elements. Thus, the emergence of this construction in NSL represents a shift from gesture-like to language-like expression.

It is informative that the first-cohort signers, who originated the language when they were children in the late 1970s, continue to produce it today in a form closer to its gestural model. We take this as an indication of the extent of their impact on NSL before the mid-1980s, when they reached adolescence. The children who were arriving in the mid-1980s then became NSL's second wave of creative learners, picking up where the first cohort left off and making changes that were never fully acquired by now-adolescent first-cohort signers (*15*, *16*). The difference today between first- and second-cohort signers therefore indicates what children could do that adolescents and adults could not. It appears that the processes of dissection, reanalysis, and recombination are among those that become less available beyond adolescence. Such an age effect is consistent with, and would partially explain, the preadolescent sensitive period for language acquisition discussed in other work (*21*, *22*). Using their early learning skills, those who were still children in the mid-1980s developed NSL into the more discrete and combinatorial system that they, and the children who followed in the 1990s, still exhibit today.

Because NSL is such a young language, recently created by children, its changes reveal learning mechanisms available during childhood. Our observations highlight two of these mechanisms. The first is a dissecting, segmental approach to bundles of information; this analytical approach appears to override other patterns of organization in the input, to the point of breaking apart previously unanalyzed wholes. The second is a predisposition for linear sequencing; sequential combinations appear even when it is physically possible to combine elements simultaneously, and despite the availability of a simultaneous model. We propose that such learning processes leave an imprint on languages—observable in mature languages in their core, universal properties—including discrete elements (such as words and morphemes) combined into hierarchically organized constructions (such as phrases and sentences).

Accordingly, these learning mechanisms should influence language emergence and change as long as there are children available to take up a language. Consistent with this account, linear sequencing of elements (even when representing simultaneous aspects of an event) appears to be an initially favored device in language emergence (*23*). For example, strong word order regularities are well documented in creoles, young languages that arise out of particular social situations of language contact (*24*–*26*). Some theories of creolization hold that child learners drive this process (*27*, *28*). Our findings, in line with these approaches, favor a degree of child influence in identifying and sequencing elements (*29*).

However, these learning predispositions will not fully determine a language's eventual structure. For example, many sign languages use simultaneous combinations in addition to sequential ones. Nonetheless, even in cases where adults use simultaneous constructions, the pattern of children's acquisition points to a preference for linear sequencing (*23*). For example, research on the acquisition of American Sign Language (ASL) (*23*, *30*) has shown that children initially break complex

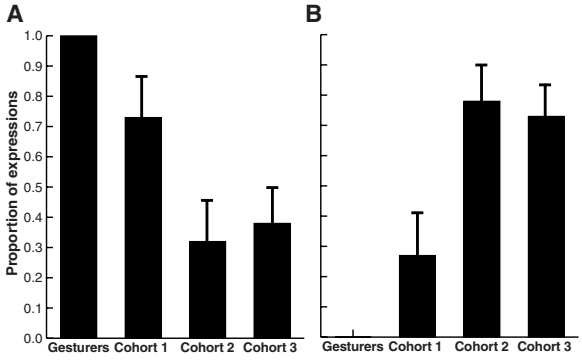

Fig. 2. (**A**) The proportion of expressions that include manner and path that articulate them simultaneously within a single gesture or sign. Proportions were computed for each participant. Bars indicate mean proportions for each of the four groups; error bars indicate SE. All of the co-speech gestures and most of the first-cohort signers' expressions articulated manner and path simultaneously. Second- and third-cohort signers produce relatively fewer expressions of this type. Proportions differ significantly across the four groups (Kruskal-Wallis, $P < 0.02$, $df = 3$, $\chi^2 = 10.8$). Post hoc analyses with Bonferroni adjustment indicate that the Spanish speakers differ significantly from second-cohort signers (Mann-Whitney, $P < 0.04$) and marginally from third-cohort signers (Mann-Whitney, $P < 0.06$). (**B**) The proportion of expressions that include manner and path that articulate them sequentially in a string of manner-only and path-only elements. Proportions were computed for each participant. Bars indicate mean proportions for each of the four groups; error bars indicate SE. These sequential expressions are never observed in the co-speech gestures. First-cohort signers sometimes produce such sequences; second- and third-cohort signers include them in most of their expressions. Proportions differ significantly across the four groups (Kruskal-Wallis, $P < 0.01$, $df = 3$, $\chi^2 = 14.7$). Post hoc analyses with Bonferroni adjustment indicate that the Spanish speakers differ significantly from both second-cohort signers (Mann-Whitney, $P < 0.02$) and third-cohort signers (Mann-Whitney, $P < 0.03$).

verb expressions down into sequential morphemes, rather than produce multiple verb elements together in the single, simultaneous movement found in adult models. In ASL, oversegmentation during acquisition was observed across a number of element types, including the agent and patient of a transitive event, and, as in NSL, the manner and path of a motion event. These elements correspond to semantic units that are relevant to lexicalization patterns in many (possibly all) languages (*20*). Thus, the elements chosen for segmentation may reveal the very primitives that children are predisposed to seek out as basic, grammatical units.

Such primitives, and the processes that isolate and recombine them, are central to children's language-learning machinery today. Whether these drove the formation of the very first human languages depends on whether languages shaped learning abilities, or vice versa. We speculate that a combination of the two was the case. Once language developed a discrete and hierarchical nature, children who tended toward analytical and combinatorial learning would have an advantage acquiring it (*3*). In this way, evolutionary pressures would shape children's language-learning (and now, language-building) mechanisms to be analytical and combinatorial. On the other hand, once humans were equipped with analytical, combinatorial learning mechanisms, any subsequently learned languages would be shaped into discrete and hierarchically organized systems (*4*, *5*).

Although our findings are consistent with both directions of effect in the evolution of learners and languages, they are at odds with accounts in which such attributes evolved externally, were passed from generation to generation solely through cultural transmission, and were never reflected in the nature of the learning mechanism (*7*). In studies of mature languages, the potential imprint of the learning mechanism is redundant with, and hence experimentally obscured by, preexisting language structure. But the rapid restructuring of Nicaraguan Sign Language as it is passed down through successive cohorts of learners shows that even where discreteness and hierarchical combination are absent from the language environment, human learning abilities are capable of creating them anew.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/305/5691/1779/DC1
Materials and Methods
Movies S1 and S2

11 May 2004; accepted 15 July 2004

Two Distinct Actin Networks Drive the Protrusion of Migrating Cells

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Cell migration initiates by extension of the actin cytoskeleton at the leading edge. Computational analysis of fluorescent speckle microscopy movies of migrating epithelial cells revealed this process is mediated by two spatially colocalized but kinematically, kinetically, molecularly, and functionally distinct actin networks. A lamellipodium network assembled at the leading edge but completely disassembled within 1 to 3 micrometers. It was weakly coupled to the rest of the cytoskeleton and promoted the random protrusion and retraction of the leading edge. Productive cell advance was a function of the second colocalized network, the lamella, where actomyosin contraction was integrated with substrate adhesion.

Cell migration involves a coordinated cycle of plasma membrane protrusion at the leading edge, adhesion site formation under the protrusion, disruption of older adhesion sites at the cell rear, and cytoskeleton contraction against adhesions to yield cell body movement (*1*). Protrusion is thought to result from actin filament (F-actin) polymerization against the plasma membrane (*2*), with the polymerization rate regulated by the rate of monomer addition to the fast-growing ("barbed") ends of filaments. This may depend on actin-related protein 2/3 (Arp2/3) complex activation, which creates free barbed ends by branching and de novo nucleation of filaments (dendritic nucleation) (*3*), and on actin depolymerizing factor (ADF) cofilin, which creates free barbed ends by severing preexisting filaments and promoting depolymerization of free filament "pointed" ends (*4*). Filament growth is limited by barbed end–capping

proteins and depletion of the polymerization-competent pool of actin monomers (*5*).

We exploited quantitative fluorescent speckle microscopy (qFSM) (*6*) to study the dynamic organization of F-actin in migrating cells and define its relationship to cell protrusive behavior. In FSM, a nonuniform pattern of fluorophores forms by copolymerization of F-actin from a pool of actin monomers with a very low ratio between fluorescently labeled and unlabeled monomers. In high-resolution images, fluorophore clusters are detected as local intensity maxima called speckles (fig. S1). We imaged speckles containing 2 to 8 fluorescent monomers. Computational tracking of $>10^5$ speckles per FSM movie (*7*) and statistical processing of their intensity fluctuations (*8*) allowed us to map F-actin flow (kinematics) and turnover (kinetics) with submicrometer resolution (*9*).

We quantified F-actin dynamics in newt lung epithelial cells (Fig. 1, A and B, and movie S1) and potoroo kidney (PtK1) epithelial cells (Fig. 1, C and D, and movie S2). In kinematic maps, both cell types exhibited a thin (1 to 3 μm) band along the leading edge where speckles underwent fast flow (300 to 500 nm/min) toward the cell center (Fig. 1, A and C, and movies S3 and S4). The band abutted a much larger area of slower retrograde flow (100 to 250 nm/min). Steady-state maps of F-actin kinetics showed a ~1-μm-wide band of strong polymerization along the leading edge that transitions into an equally narrow band of depolymerization (Fig. 1, B and D), which corresponded in kinematic maps to the region of negative speed gradients. Animated kinetic maps (movies S5 and S6) revealed that bursts of depolymerization also occurred at the leading edge. Beyond these bands, kinetic maps displayed a random pattern of foci 1 to 2 μm in diameter, where F-actin assembled and disassembled in cycles of ~40 to 50 s. We refer to the zone with fast retrograde flow and juxtaposed narrow bands of polymerization and depolymerization as the lamellipodium and the zone characterized by slower flow and a punctate pattern of filament turnover as the lamella. The lamellipodium and lamella had distinct molecular signatures, with high concentrations of Arp2/3 and ADF/cofilin in the lamellipodium and myosin II and tropomyosin present exclusively in the lamella (fig. S2).

Given the kinematic, kinetic, and molecular differences, we sought mathematical criteria that would allow us to determine the relationship between the lamellipodium and lamella over time. We tracked the cell border and calculated the rates of network assembly, disassembly, and retrograde flow along regularly spaced profiles perpendicular to the edge (Fig. 1E). In 80 profiles analyzed, the assembly rate peaked at ~1 μm from the leading edge, followed closely by a peak in depolymerization (Fig. 1F). Before the variation in both rates diminished, a second peak of net assembly (Fig. 1F, arrowhead) indicated the location where lamellipodium kinetic behavior turned into lamella kinetic behavior. This kinetic boundary colocalized with the first local minimum of the retrograde flow speed (Fig. 1G). Together, these criteria permitted robust automatic extraction of the lamellipodium-to-lamella transition (Fig. 1E, white line).

Integration of the net assembly rate (the difference between areas A and D in Fig. 1F) revealed the amount of polymer generated and disassembled within the boundary of the lamellipodium. Most (85 to 90%) of the actin network underwent a complete cycle of assembly and disassembly within the first 1 to 3 μm from the cell edge. Thus, lamellipodium and lamella are composed of materially nearly decoupled networks, suggesting that the lamellipodium does not supply substantial filaments to other actin assemblies in the cell (*10*). The tight spatial juxtaposition of assembly and disassembly likely generates monomer gradients high enough to maintain an efficient treadmill

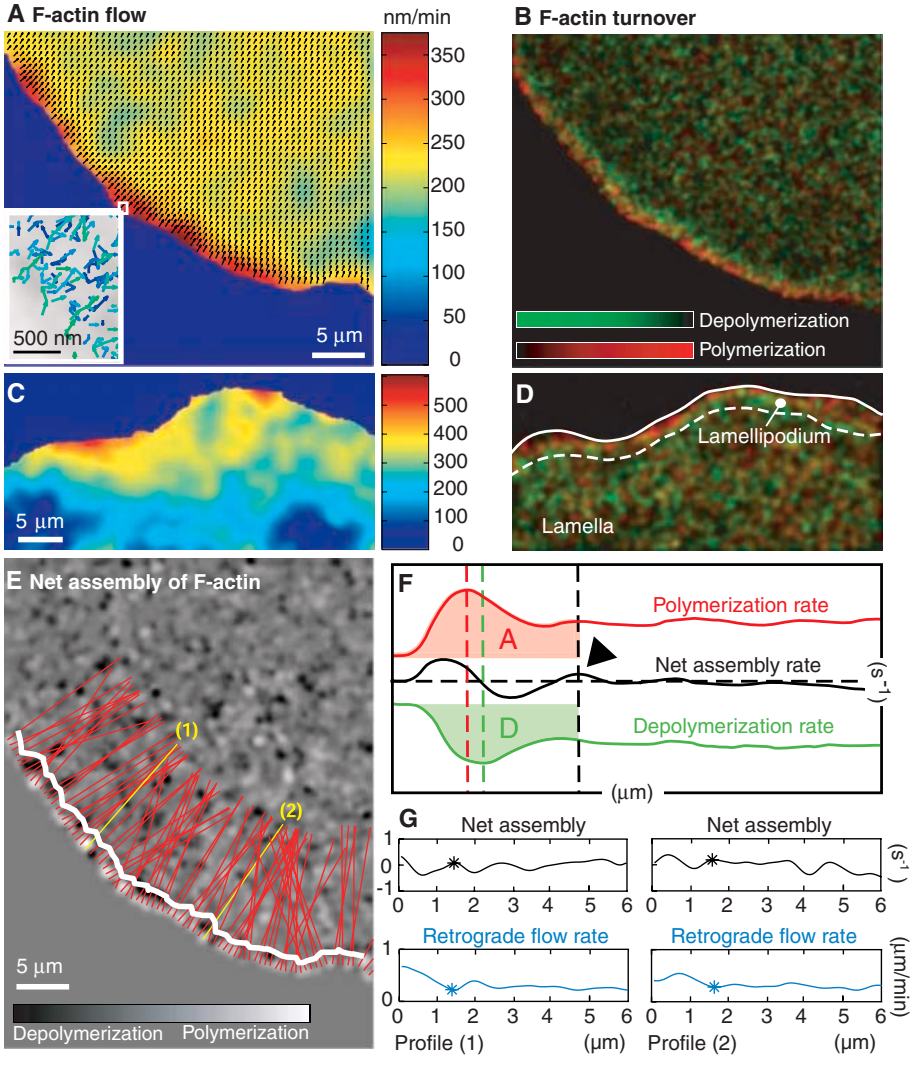

Fig. 1. F-actin dynamics characterized by qFSM in (**A** and **B**) a newt lung cell (movies S1, S3, and S5) and (**C** and **D**) a PtK1 cell (movies S2, S4, and S6). [(A) and (C)] Time-averaged F-actin flow (60 frames); colors encode flow speed, and vectors, flow direction. Inset: Individual speckle trajectories over 200 s; time evolution, dark blue to light green. [(B) and (D)] Time-averaged F-actin turnover (60 frames). Red channel, assembly rate; green, disassembly rate. (**E**) Net F-actin assembly. Black, negative assembly rates; white, positive assembly rates. The lamellipodium-to-lamella transition (white line) (movie S8) was computed from rate profiles (along the red lines) of net assembly and flow. (**F**) Rate profiles indicate that the lamellipodium-to-lamella transition coincides with the first local maximum of net assembly (arrowhead) after the first maximum in depolymerization (dashed green line). A, integrated assembly; D, integrated disassembly. (**G**) The kinetically defined transition (black asterisks) colocalizes with the first local minimum in flow rate (blue asterisks). Examples are calculated from the yellow profiles (1) and (2) in (E).

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of actin turnover based on diffusive transport (*11*–*13*).

Next, we partitioned speckles into two classes (Fig. 2A): fast-moving and short-living (class 1) and slow-moving and long-living (class 2). Class 1 speckles clustered in the lamellipodium, whereas 90% of the speckles in the lamella belonged to class 2 (Fig. 2B). However, 33% of the speckles falling into the area between the leading edge and the lamellipodium-lamella transition had the characteristics of lamella speckles (Fig. 2B, inset). We examined the ensemble behavior of these speckle classes and found that class 1 speckles displayed the kinetic signature of the lamellipodium (Fig. 2C), whereas class 2 speckles displayed the signature of a lamella network (Fig. 2D) that extended all the way to the leading edge. This coexistence of two spatially overlapping yet kinetically and kinematically distinct networks was masked in joint evaluation of all speckles (Fig. 1, B and D), because class 1 speckles outnumbered class 2 speckles near the cell boundary.

We probed the molecular characteristics of the two networks in a series of pharmacological perturbation experiments. First, we applied blebbistatin to inhibit nonmuscle myosin II adenosine triphosphatase activity (*14*). In agreement with myosin II localization data (fig. S2), blebbistatin reduced lamella retrograde flow but not lamellipodium flow (Fig. 3A), with little effect on the rates of filament turnover in either network.

We next treated cells with 0.5 μM cytochalasin D (cytD) (Fig. 3B) to inhibit polymerization of free barbed ends (*15*). Spacetime diagrams of F-actin turnover, edge displacement, and network flow computed along the leading edge (Fig. 3C) (*9*) revealed that, before cytD perfusion, periodic bursts of polymerization at the leading edge transformed in equal parts into protrusion and F-actin retrograde flow (Fig. 3C, dashed white lines). Cross-correlation analysis of the three parameters (Fig. 3D) confirmed a positive correlation between edge displacement and network turnover and negative correlations for the two other parameter combinations. This behavior changed after cytD perfusion. The correlation between turnover and both edge displacement and network flow broke down, and the correlation between edge displacement and network flow switched from negative to positive values (Fig. 3D). Thus, cytD selectively removed the lamellipodium network by capping the free barbed ends, and the synchronous retraction of the leading edge and network (movie S7) is associated with lamella contraction (*16*). Despite cytD treatment, the lamella was in a state of predominant disassembly for only ~6 min (disassembly:assembly ratio of 1.8 ± 0.2). Subsequently, periodic patterns of assembly and disassembly were re-established (disassembly:assembly ratio of 1.2 ± 0.1 in cytD versus 1.4 ± 0.1 before perfusion), indicating that much of the kinetic activity in the lamella network is insensitive to cytD. However, the maintenance of the lamella position and its advancement required barbed end assembly. Washout of cytD immediately re-established the leading edge position and lamellipodial kinetic and kinematic signatures.

Similarly, inhibiting depolymerization by stabilizing filaments with 1 μM jasplakinolide (*11*) also affected the lamellipodium selectively. Within minutes of jasplakinolide application, both filament turnover and retrograde flow were stopped in the lamellipodium, whereas the lamella appeared unaffected (Fig. 3E). The loss of the lamellipodium did not alter edge position, and the same region now showed the signatures of the lamella, providing additional evidence for two distinct yet spatially overlapping networks.

Which of the two networks drives cell protrusion? We compared leading edge displacement, network turnover, and retrograde flow in movies of cells that displayed very slow net advancement to those with persistent rapid protrusion. For cells with slow edge advancement, without exception, we found periodic cycles of edge protrusion and retraction (*17*). They correlated with cycles of polymerization and depolymerization and of faster and slower retrograde flow. F-actin flow in the lamella (measured proximal to the lamellipodium-lamella transition) exhibited a periodicity highly correlated with the lamellipodium flow (Fig. 4A), but the amplitude modulation was damped by 69% (fig. S4), confirming the rather weak material coupling between the two networks.

Next, we examined cells with rapid, persistent edge protrusion (Fig. 4B and movie S2). One possibility was that the balance between forward edge movement and lamellipodium retrograde flow could be tipped toward edge movement. However, when polymerization increased (movie S6), it was still accompanied by acceleration of retrograde flow (movie S4). Alternatively, propulsion of the leading edge by increased polymerization could be accompanied by a widening of the lamellipodium. Instead, we found that the cell boundary and lamellipodium-to-lamella transition advanced in concert (Fig. 4C), creating the impression of a lamellipodium surfing on a forward-growing lamella (movies S8 and S9). Thus, persistent protrusion depends on the local expansion of the lamella network. Lamellipodium polymerization is not sufficient.

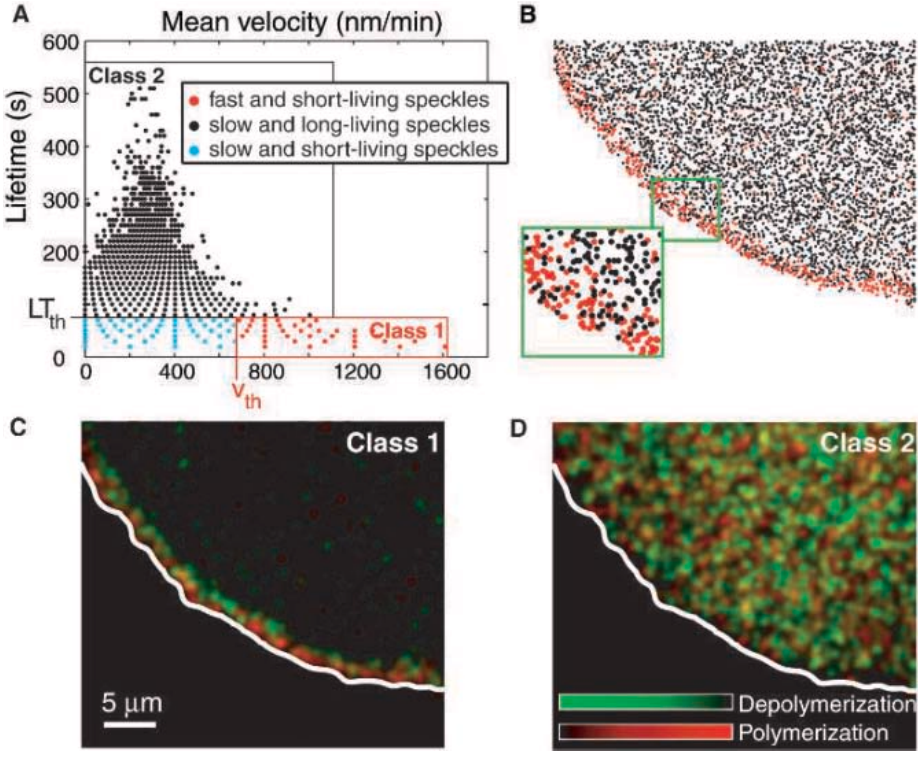

Fig. 2. Distinction of spatially overlapping lamellipodium and lamella by classification of speckle velocity and lifetime. (**A**) Separation of fast-moving and short-living speckles (class 1, red) and slow-moving and long-living speckles (class 2, black). LT_{th}, lifetime threshold; V_{th}, velocity threshold. (**B**) Thresholds are set to minimize the number of class 1 speckles in the lamella while maximizing the number in the lamellipodium. This multi-objective optimization has a unique solution, because class 1 speckles cluster in the lamellipodium with a residual occurrence of ~10% in the lamella. Class 2 speckles dominate the lamella but expand all the way to the leading edge, contributing 33% to the lamellipodium region. (**C**) Turnover calculated from class 1 speckles shows the spatial kinetic signature of the lamellipodium. (**D**) Turnover calculated from class 2 speckles shows the spatial kinetic signature of the lamella.

To determine if the expansion of the lamella spatially correlated with the establishment of linkages between the cytoskeleton and the extracellular matrix, we filmed actin dynamics by FSM and localized focal adhesions using green fluorescent protein (GFP)-tagged vinculin. Protrusive edge sections (Fig. 4D, arrowheads) were consistently positioned just beyond the distalmost portion of focal adhesions where the adhesions initiated at the lamellipodium-to-lamella transition.

Thus, qFSM of F-actin dynamics in migrating cells reveals two kinetically, kinematically, and molecularly distinct F-actin networks at the leading edge of epithelial cells, which spatially overlap and yet are only weakly coupled, and whose transition may be defined by the initiation of substrate-cytoskeleton linkages (fig. S5). Productive advancement of the leading edge requires expansion of the lamella, where actomyosin contractile forces are coupled to substrate adhesion and which depends on cytD-insensitive filament assembly, possibly mediated by formins (*18*, *19*). In contrast, lamellipodium protrusion and retraction probably serve an exploratory function or could provide rapid responses to extracellular cues. However, persistent advancement of the cell relies on the underlying lamella.

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20. Supported by research grant no. RGY5-2002 from the Human Frontiers Science Programme and no. GM67230 from the National Institutes of Health. This is TSRI manuscript no. 16575-CB.

Supporting Online Material

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Materials and Methods

Figs. S1 to S5

Movies S1 to S9

20 May 2004; accepted 6 August 2004

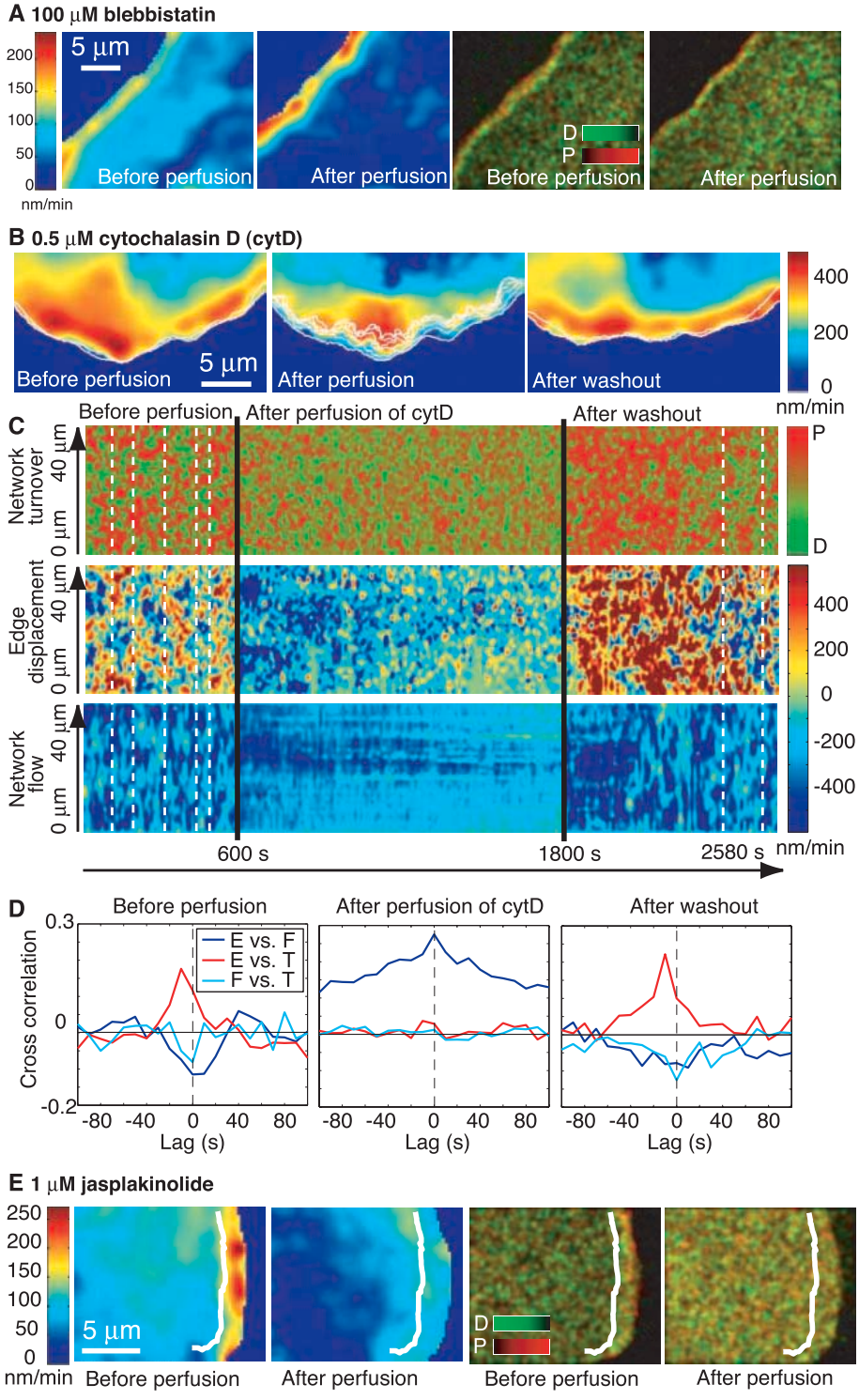

Fig. 3. Probing molecular characteristics of the two networks by small molecule inhibitors. (**A**) Perfusion with 100 μM blebbistatin slows lamella F-actin flow exclusively (left two panels), with little effect on F-actin turnover (right two panels). P, polymerization; D, depolymerization. (**B**) Perfusion with 0.5 μM cytD decreases F-actin flow in the lamellipodium and induces retraction of the leading edge (white lines) (fig. S3). Washout rescues cell edge position and lamellipodium flow rate. (**C**) Space-time diagrams of F-actin network turnover, edge displacement, and F-actin flow along the leading edge (fig. S3) (*9*) during perfusion and washout of cytD. White dashed lines highlight periodicities. (**D**) Correlation between edge displacement (E) and F-actin flow (F); between edge displacement and F-actin turnover (T); and between flow and turnover. (**E**) Perfusion with 1 μM jasplakinolide selectively removes the kinetic and kinematic signature of the lamellipodium. The white line indicates the lamellipodium-to-lamella transition before drug application.

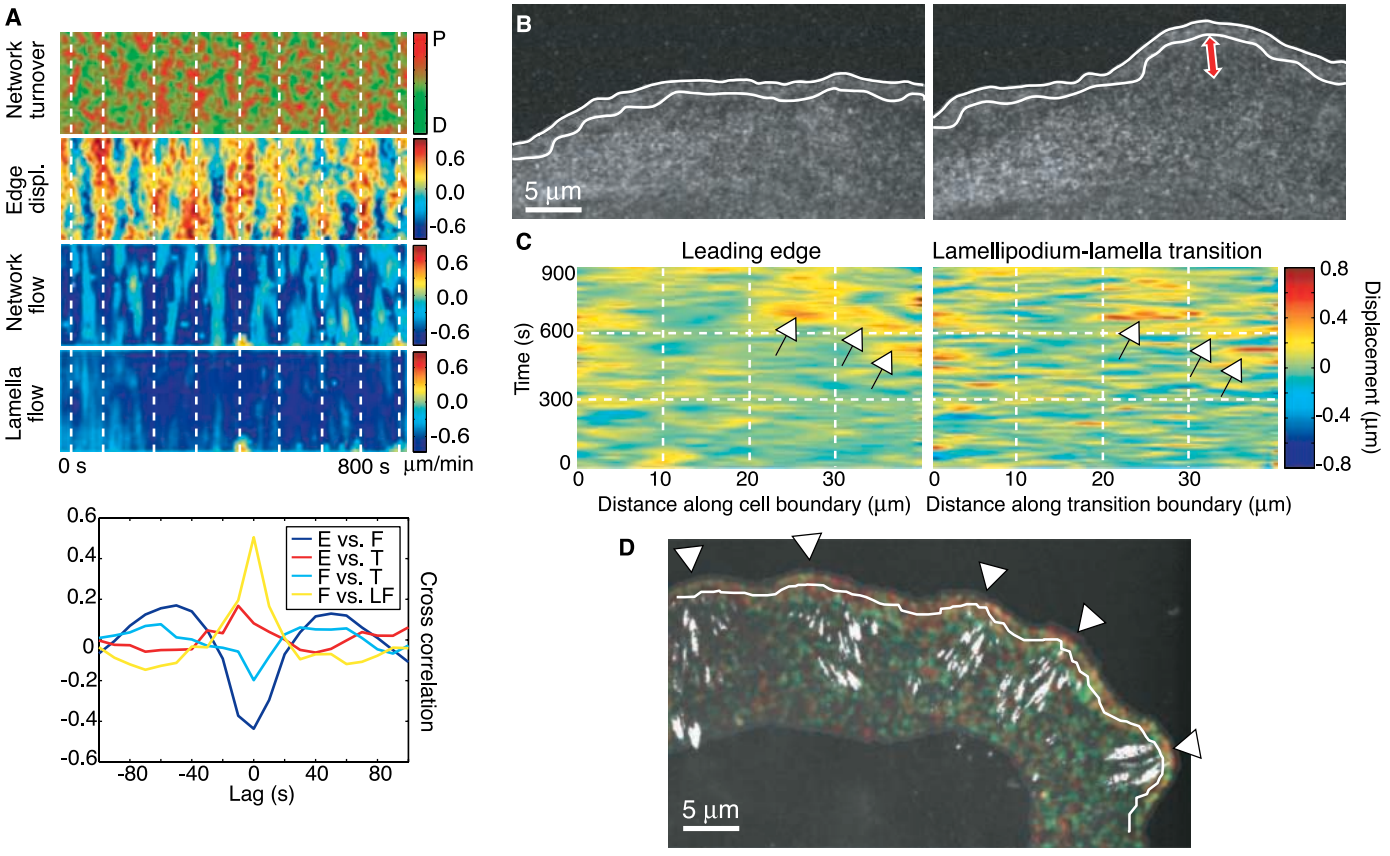

Fig. 4. The contribution of lamellipodium and lamella to edge protrusion. (**A**) Space-time diagrams of F-actin turnover, edge displacement (displ.), and F-actin flow along the leading edge and lamella F-actin flow adjoining the lamellipodium-to-lamella transition (fig. S4). Cycles of assembly and disassembly at the leading edge (period, 0.01 Hz) are transformed in equal parts into oscillatory edge movement and retrograde flow (white dashed lines). The same period is found in lamella flow (there is a high correlation between flow at the leading edge and lamella flow, F versus LF), but the amplitudes are markedly decreased (cf. fig. S4). (**B** and **C**) Co-movement of the leading edge and the lamellipodium-to-lamella transition during persistent cell protrusion [the red arrow in (B) and arrows in (C)] (movie S9). (**D**) Overlay of GFP-vinculin (white), marking focal adhesions on the actin turnover map and showing protrusions of the leading edge (arrowheads) and the position of the lamellipodium-lamella transition (white line).

Zooming In on a Quantitative Trait for Tomato Yield Using Interspecific Introgressions

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To explore natural biodiversity we developed and examined introgression lines (ILs) containing chromosome segments of wild species (*Solanum pennellii*) in the background of the cultivated tomato (*S. lycopersicum*). We identified *Brix9-2-5*, which is a *S. pennellii* quantitative trait locus (QTL) that increases sugar yield of tomatoes and was mapped within a flower- and fruit-specific invertase (*LIN5*). QTL analysis representing five different tomato species delimited the functional polymorphism of *Brix9-2-5* to an amino acid near the catalytic site of the invertase crystal, affecting enzyme kinetics and fruit sink strength. These results underline the power of diverse ILs for high-resolution perspectives on complex phenotypes.

The genetic basis of many natural phenotypes takes the form of a continuous range rather than discrete classes. The complexity of traits showing continuous distribution often results from the segregation of numerous QTL, whose expression is modified both by the environment and by genetic background (*1*). Genetic resolution of quantitative traits in populations that segregate simultaneously for different QTL scattered throughout the genome [e.g., second filial generation (F2), backcross, and recombinant inbreds] is low compared with QTL analysis in lines that segregate for a single region (i.e., ILs in plants and congenic strains in animals) (*2*). Multiple segregating QTL at the whole-genome level tend to mask the effects of one another by introducing high variances in statistical analyses. In sharp contrast, ILs are identical for the entire genome except for a single introgressed region, and therefore all the phenotypic variation in these lines is associated with the introduced segment. The use of such targeted population structures increased the identification power for

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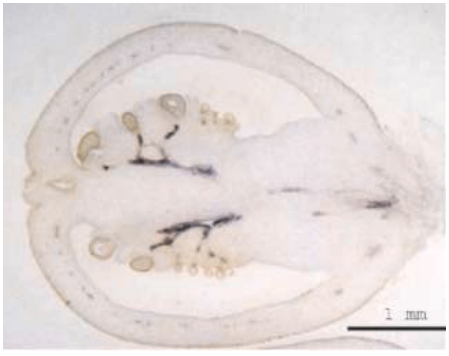

Fig. 1. *LIN5* expression in the conductive tissues of the developing tomato fruit. In situ localization of *LIN5* transcripts in a longitudinal section of an ovary at anthesis showing expression mainly in the conductive tissues within the placenta that lead to the developing seeds as well as the surrounding pericarp.

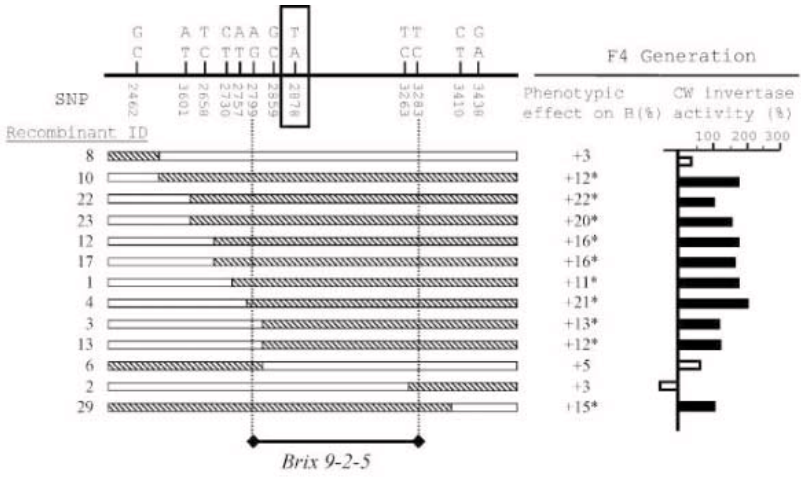

Fig. 2. Map of the effect of *Brix9-2-5* on B and on CW invertase activity. Each recombinant is shown by a combination of empty and hatched bars, representing the *S. lycopersicum* and *S. pennellii* sequence, respectively. Each SNP is represented by its position in the *S. pennellii* (top) and *S. lycopersicum* nucleotides. The SNP^{2878}, which underlines the QTN effects, is boxed. Phenotypic effects of each recombinant family's alleles on the B and the CW invertase activity is shown as the percentage difference from the *S. lycopersicum* line. Full bars and asterisks denote a significant difference from the control ($P < 0.001$).

QTL by several times in both plants and animals (*3*, *4*).

QTL cloning projects in plants have focused primarily on variation derived from naturally divergent genetic resources and have generated new biological insights about plant development, whereby some of the effects could be delimited to single-nucleotide alterations (*5*–*8*). Biodiversity research in crop plants has shown that when chromosome segments of wild species are introgressed into cultivated varieties, it is possible to identify genomic regions that substantially improve yield (*9*). To enhance the progress of tomato breeding, we developed a population of 76 segmental ILs that are composed of marker-defined genomic regions of the wild species *S. pennellii* (accession code LA716), substituting for the homologous intervals of the cultivated variety M82 [*S. lycopersicum*; the taxonomic classification of tomato in the genus *Solanum* is available in (*10*)]. The ILs that represent the entire genome partition the genetic map into 107 bins, which are defined by singular or overlapping segments (*11*). Over the past 10 years, the tomato ILs have been assayed for yield-associated traits and the data are presented, in silico, in a search engine that displays the components of the genetic variation (*12*).

The QTL database creates a firm basis to investigate the genetic control of yield-associated traits. An important yield component in "ketchup tomatoes" is the total soluble content of the fruit measured in refractometer brix units (consisting mainly of the sugars glucose and fructose). The *S. pennellii* IL data for brix (B) show 25 different genomic regions with significant effects on the trait relative to M82; in the majority of these cases, the *S. pennellii* alleles increased B (fig. S1). To uncover the molecular control of B, we characterized the QTL *Brix9-2-5*, the effects of which ranged from an increase of 11 to 25%, depending on genetic background and environmental factors (*13*, *14*). This moderate QTL improves B without reducing total yield, thus increasing the sugar output per unit area, which is a key yield parameter in industrial tomatoes (*9*). High-resolution genetic mapping delimited *Brix9-2-5* to a single-nucleotide polymorphism (SNP)–defined region of 484 base pairs spanning part of the third exon and the third intron of the cell-wall (CW) invertase (*LIN5*) (*14*). Another component of the complexity in the molecular analysis of this gene is that *LIN5* is a member of a larger gene family including three additional CW invertases (*LIN6*, *LIN7*, and *LIN8*). After investigating the RNA profile of the *LIN* family, we concluded that the window of exclusivity of *LIN5* expression is in the conductive tissue of the flower's ovary, fruit placenta, and pericarp during the early stage of cell division (*15*) (Fig. 1). The expression of *LIN5* in the conducting tissue next to a potential "sugar-unloading site" near the ovaries is consistent with the role of *LIN5* as a "sink gene" that regulates the ability of the fruit to import photosynthetic sugars (*16*). Because of the partially dominant nature of the *S. pennellii* allele in elevating fruit B (*14*), we compared transcription rates of the wild and cultivated species alleles in young ovaries of heterozygous plants. Steady-state mRNA levels of the *S. pennellii* allele relative to the *S. lycopersicum* allele were statistically similar (fig. S2), indicating that the QTL effect was not due to differential transcription modulation by the third intron (*14*). To compare the quantities of the *LIN5* protein, we extracted the CW-bound enzymes from the ovules of the *Brix9-2-5* nearly isogenic lines and analyzed them on protein gel blots with antibodies raised against CW invertase (*17*). The lines did not differ statistically in the mean quantity of *LIN5* peptide (fig. S3). However, CW invertase activity was statistically different ($P < 0.01$) between the genotypes, with values three to five times as high in lines homozygous for the *S. pennellii* allele compared with those of the *S. lycopersicum* lines (Table 1).

The fact that the IL9-2-5 differed from the control for enzyme activity in flowers did not mean that these differences map to the same *LIN5* interval as the fruit sugar QTL, which is manifested 50 days after flowering. An example for the complexity of the genetic factors that regulate quantitative traits was revealed for *Drosophila* alleles of alcohol dehydrogenase of differing activity, which is controlled by at least three different zones of the gene (*18*). To evaluate whether the differences in CW invertase activity were associated with the 484–base pair B QTL interval, we assayed the high-resolution recombinant families that span the gene (Fig. 2). The mapping analysis demonstrates that mature fruit B and CW invertase activity in flower ovaries are associated with the same *LIN5* interval. The data presented so far imply that the three amino acid differences in the third exon are responsible for the QTL effects (Fig. 3A).

Interspecific advanced backcross breeding populations in tomato involving *S. pimpinellifollium*, *S. habrochaites*, *S. neorickii*, and a different accession of *S. pennellii* (LA1657) from that used for the IL construction were next assayed for yield-associated traits (including B) in three to seven locations. *Brix9-2-5* maps to the middle of the short arm of

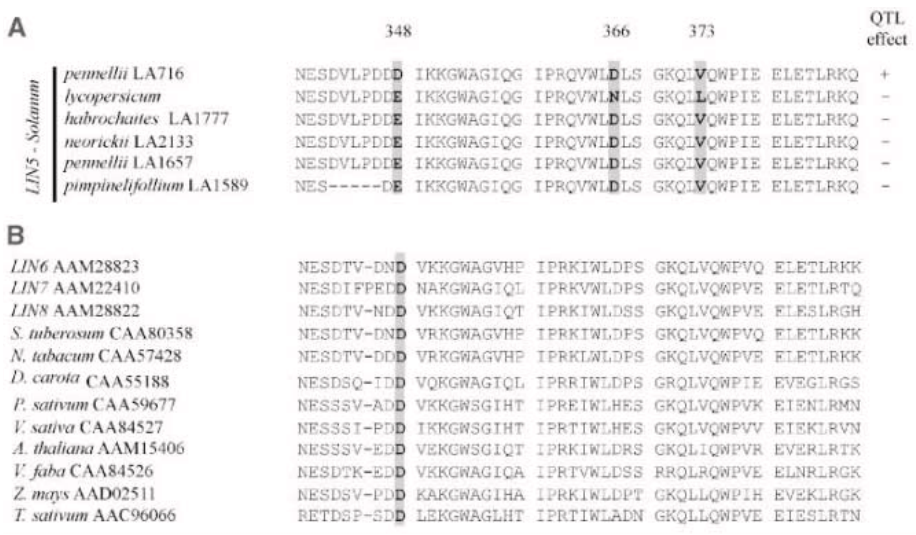

Fig. 3. Alignment of the *Brix9-2-5* peptide sequence (*28*). (**A**) Tomato species *Brix9-2-5* allelic series. The relevant three amino acid substitutions between *S. lycopersicum* and *S. pennellii* are in bold. The phenotypic effect (+, present; –, absent) of the wild species' alleles located along the short arm of chromosome 9 on B is indicated on the right and is based on multiple-site testings of advanced backcross populations (*18–21*). (**B**) Sequence alignment of the protein region spanning *Brix9-2-5* in several plants showing a conserved aspartate (D).

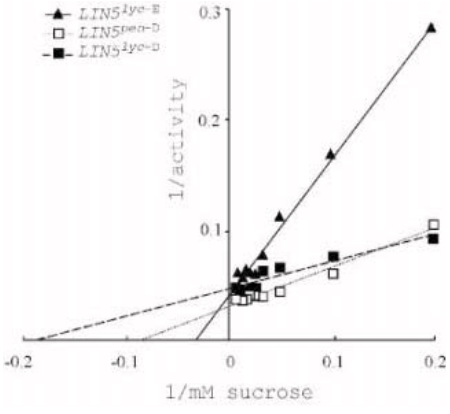

Fig. 4. Biochemical analysis of invertase-deficient yeast complemented by *LIN5* alleles. Kinetic analysis of the *LIN5* enzymes from yeast strains overexpressing the proteins from *S. lycopersicum* ($LIN5^{lyc\text{-}E}$), IL9-2-5 ($LIN5^{pen\text{-}D}$), and mutated *S. lycopersicum* ($LIN5^{lyc\text{-}D}$). Acid invertase activity was measured in soluble fractions of a 3-day yeast culture with different substrate (sucrose) concentrations. Each point represents a mean of four replicates.

Table 1. IL9-2-5 effects on invertase activity in flowers. Invertase activity (mmol reducing sugars per gram fresh weight per hour) was determined in the insoluble fraction of ovaries before anthesis and during anthesis. Mean values were calculated based on five replicates of 10 ovaries each (±TE).

Genotype/stage	Cell wall invertase activity	
	Preanthesis	Anthesis
M82	9.5 ± 4.1	20.4 ± 4.1
IL9-2-5	32.0 ± 4.1	63.2 ± 4.1

chromosome 9; none of these populations revealed a B QTL on the short arm of chromosome 9, in any of the sites tested (*19–22*) (Fig. 3A). Sequencing of the QTL region of *LIN5* from the above accessions showed that they all share the Asp^{366} and Val^{373} residues with *S. pennellii* (LA716). However, Asp^{348} was uniquely associated with LA716 (Fig. 3A). Interestingly, multiple alignment of *LIN5* with its duplicated family members (*LIN6*, *LIN7*, and *LIN8*) and other plant invertases exposed aspartate as a conserved residue at this position (Fig. 3B), suggesting a nonneutral role for this amino acid.

The candidate quantitative trait nucleotide (QTN) (SNP^{2878}; Fig. 2) responsible for the Asp^{348} substitution was evaluated in complementation tests in an attempt to study its effects on enzyme activity. We used a yeast invertase–deficient strain that completely lacks the ability to degrade sucrose (*23*) as a host for plasmids harboring the tomato *LIN5* cDNA from *S. lycopersicum* ($LIN5^{lyc\text{-}E}$, where the letter in the superscript depicts the identity of the amino acids at positions 348), IL9-2-5 ($LIN5^{pen\text{-}D}$), and the mutated *S. lycopersicum* allele engineered to code for an invertase with the Asp^{348} residue ($LIN5^{lyc\text{-}D}$). Unlike the empty-vector control transformants, the three invertase-transformed yeast strains exhibited substantial growth on sucrose, with $LIN5^{pen\text{-}D}$ and $LIN^{lyc\text{-}D}$ alleles seemingly complementing the invertase deficiency of the yeast strain more faithfully than did the $LIN5^{lyc\text{-}E}$ allele (fig. S4). These differences could not be attributed to changes in the quantity of the enzyme because immunoblot quantification did not reveal significant differences in the total amount between the three different yeast genotypes (fig. S5). When sucrose hydrolysis activities of the expressed invertases from the three strains were plotted as a function of substrate concentration, the double reciprocal plots were linear (Fig. 4) and the Michaelis constant for sucrose ($Km_{[sucrose]}$) values calculated from these plots are 31.0, 11.6, and 5.1 mM for $LIN5^{lyc\text{-}E}$, $LIN5^{pen\text{-}D}$, and $LIN5^{lyc\text{-}D}$ alleles, respectively. The values for the *S. pennellii* and the mutated *S. lycopersicum* enzymes were similar to those previously reported for plant invertases, but the nonmutated *S. lycopersicum* was considerably less efficient than the values documented in the BRENDA database (www.brenda.uni-koeln.de). The complementation data indicate that Asp^{348} plays a role in enhancing the activity of *LIN5* and establish that SNP^{2878} (Fig. 2) is the QTN responsible for this phenotype as well as for fruit sugar yield. The recent resolution of the three-dimensional structure of *Thermotoga maritima* invertase revealed a bimodular arrangement of the protein with a catalytic active site that has a pocket topology (*24*). The *S. pennellii* Asp^{348} aligns with the amino acid Tyr^{266} in *T. maritima*, which is within 10 Å of the catalytic site that is likely associated with sucrose recognition and binding. The results presented in our QTL study appear to be consistent with the crystal structure analysis, thus providing a clue about how a point mutation can alter tomato invertase kinetics and sugar yield in a quantitative way.

The present study relies on the high-resolution QTL mapping attributes of the multispecies tomato IL resource and presents a zoom-in view of a QTN, from the whole-genome perspective to a single functional amino acid in the crystal structure. In *Drosophila*, which is a model organism for the dissection of complex traits, most of the polymorphisms affecting quantitative variation are in putative regulatory regions that tend to fractionate into multiple linked QTL upon high-resolution analysis (*25*). This is in contrast to *Brix9-2-5* and other plant QTL, which in most cases maintain their distinct "single-factor" identity in fine mapping (*5*). It is too early to tell whether the difference in the genetic architecture of quantitative traits between *Drosophila* and plants reflects different biology or relates to the amount of diversity between the parents that were used in the analyses. The cultivated and wild species that parented the ILs are highly divergent in sequence, but more importantly in phenotype, thus providing abundant segregation for naturally selected variation affecting yield, morphological, and biochemical traits. For ILs, chromosome substitution strains (*4*), and other permanent populations, resource construction must be followed by the much more daunting task of phenotyping of different traits over varied environments and

genetic backgrounds (*26*, *27*). Such QTL databases, housing hundreds of phenotypes measured on a common set of ILs, will lead to association between seemingly unrelated traits and allow us to explore higher level organization of complex phenotypes and the role of pleiotropy.

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28. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

29. We thank L. Willmitzer for support and discussions, T. Pleban for the technical assistance, A. Sturm for the invertase antibody, A. G. Heyer for advice on yeast transformations, and D. Spooner for updating the new tomato taxonomy. We are grateful to Y. Eshed, E. Pichersky, and S. Wolf for the helpful discussions. This research was supported by a grant from the German-Israeli Cooperation Project (DIP).

Supporting Online Material

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18 June 2004; accepted 5 August 2004

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Presenter: Lee Ducat
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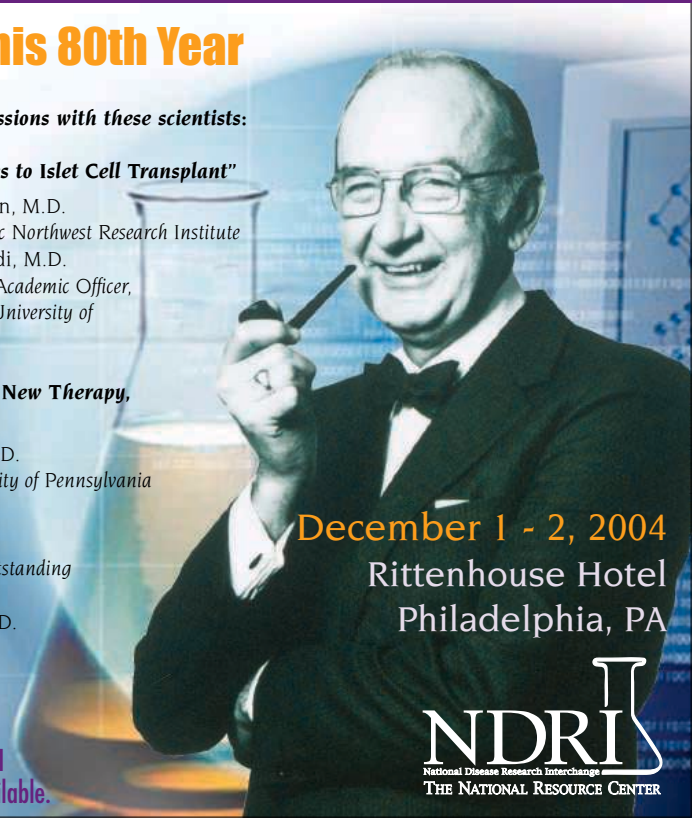

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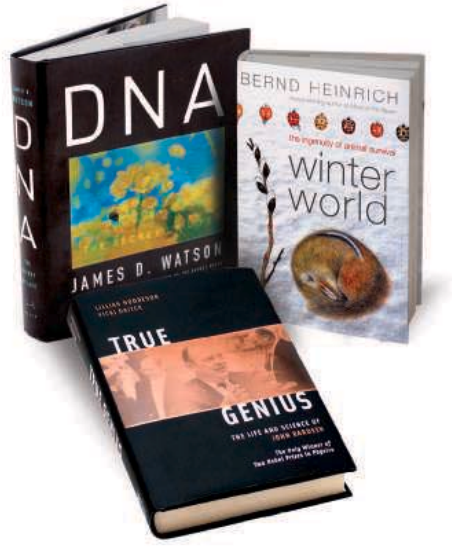

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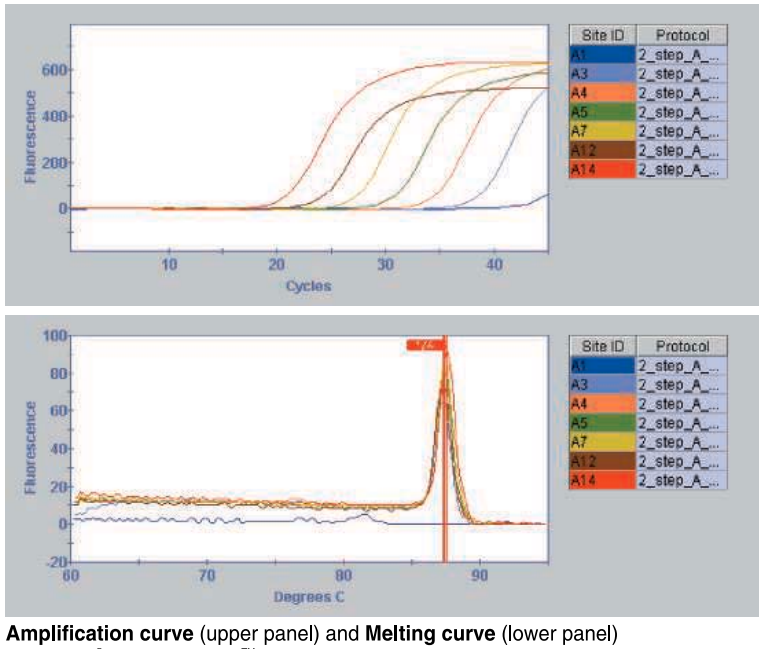

Amplification curve (upper panel) and **Melting curve** (lower panel) for SYBR® *Premix Ex Taq*™ (Perfect Real Time).

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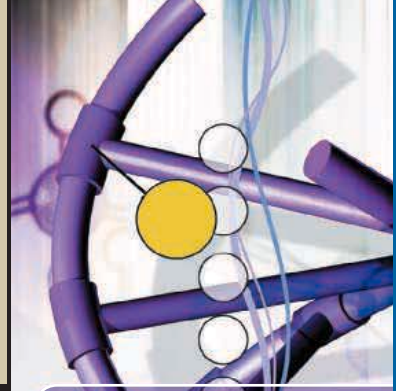
Drug Discovery and Biotechnology Trends

Genomics 3: It's a Knockdown!



In this issue:

- Gene knockout systems
- siRNAs
- siRNA libraries
- Isolation and characterization of siRNA
- Transfection methods for cells
- Cell signaling
- Target identification and validation
- Therapeutic applications of siRNA

With its ability to silence genes, RNA interference has made a loud noise in life science laboratories. Researchers have started to apply it in areas as diverse as research on cell signaling, drug discovery, and human therapeutics. BY PETER GWYNNE AND GARY HEEBNER

When scientists introduce segments of double-stranded RNA into cells, the segments break up into strands of RNA 21 to 25 base pairs in length. Those "small interfering RNA" (siRNA) strands cause RNA interference, a natural phenomenon that blocks genes from being expressed inside a living cell. Referred to as RNAi, the effect plays a key role in antiviral defense, gene regulation, and genomic rearrangements in plants, insects, and mammals.

Within the past few years, RNAi has taken the world of life science by storm, becoming an essential tool for both fundamental and applied research. "RNAi is the hottest technology in biotechnology today; it has the potential to revolutionize biology research and drug target discovery," says Jean-Louis Roux dit Buisson, CEO of **Elchrom Scientific**. "I can't think of a single biological area, in industry, academe, or government, that's not using RNAi," says David Brown, senior scientist and head of the siRNA project group at **Ambion**. "I've heard of several instances where reviewers comment that papers should be validated by RNAi." Christophe Echeverri, lead founder, CEO, and chief scientific officer of **Cenix BioScience**, agrees. "It's changing everything," he says. "We're seeing the beginning of the era of RNAi-based functional genomics."

METHOD OF CHOICE

Jim Hagstrom, vice president of scientific operations at **Mirus Bio Corporation**, explains why scientists have welcomed the technology so heartily. "RNAi has become the method of choice for looking at the cause and effect of an individual gene's expression as it relates to other genes," he says. "The impact has been enormous," adds Ron Herzig of **Upstate**. "For the first time, scientists have been able to determine the function of a gene very quickly. This technology made it very easy and economical to accomplish that."

RNAi has developed with remarkable speed. "The start came in the late 1990s in a paper by Craig Mello and Andrew Fire [of the University of Massachusetts Medical School and The Carnegie Institute, respectively] on the use of double stranded DNA for gene silencing," says Echeverri. "But the big explosion happened when Tom Tuschl, then at the Max Planck Institute in Göttingen, Germany and now at Rockefeller University, demonstrated a way to make it work in human cells." The emergence of the approach surprised much of the life science community. "Nobody really expected that such fundamental new mechanisms were still to be discovered," says Nagesh Mahanthappa, director of corporate development at **Alnylam Pharmaceuticals**. "We've probably only seen the tip of the iceberg yet."

Suppliers of RNAi-related tools, technologies, and reagents have furthered the advance of the

The companies in this article were selected at random. Their inclusion in this article does not indicate endorsement by either AAAS or Science, *nor is it meant to imply that their products or services are superior to those of other companies.*

This is the third of four supplements this year on genomics. The first two appeared in the 6 February and 9 April issues of Science. *The last will appear in the 22 October issue.*

MORE ›››

approach. "They realized that it worked and then made it available to researchers and drug companies by providing tools off the shelf," says Hagstrom. "That raised awareness of the technology; it created the buzz."

Vendors also improved the basic tools. "They have contributed most notably in the chemistry of siRNA. They have driven the advancement of siRNA's purity, stability, and design, and have minimized off-target effects," Herzig says. "The siRNA design tools available free on many academic sites give you about 70 percent success," adds Walter Tian, business director of **Qiagen**. "Commercial companies such as Qiagen have developed much better computer-generated design algorithms that give you up to 90 percent efficiency." Commercial suppliers also emphasize user friendliness. "They have brought a level of standardization so that your laboratory doesn't have to start from scratch," says Joel Hansen, application development director at **NanoDrop Technologies**.

SUPPRESSING GENES

RNAi's value stems from its ability to suppress genes – a capability also called knocking down, or silencing genes. Doing that enables scientists to focus on the function of individual genes. RNA interference isn't the first technology to achieve that goal. **Charles River Laboratories**, **The Jackson Laboratory**, and **Taconic**, among other organizations, offer knockout mice, in which specific genes have been completely suppressed. **ArtisOptimus** supplies primary mouse embryo fibroblasts from a large variety of knockout and transgenic mice. Altered tissue culture cells with knocked out genes are also available. And several researchers have used antisense technology to dampen down the functions of genes.

However, RNAi shows significant advantages over those older approaches. Thus, while knockout systems suppress genes entirely, RNAi reduces their expression level – a simpler and faster approach for basic understanding of gene function. On the other hand, RNAi silences genes more effectively than antisense technology. "Antisense has never been broadly adopted. It's too hard to do, it takes too long to optimize, and it causes too many undesired side effects," explains Tian of Qiagen. "RNAi is much more efficient than the antisense oligos in terms of getting knockdown in vivo," Hagstrom adds.

Bill Marshall, executive vice president research and operations at **Dharmacon**, summarizes RNAi's benefits. "The ease of implementation, time to receive a quality answer, and the robustness of the technology are all optimal with RNAi compared with past technologies," he says. "It's probably the most enabling reverse genetics tool that the scientific community has ever encountered."

Methods of producing siRNAs include chemical synthesis, in vitro transcription, digestion of long double stranded RNA (dsRNA) by a so-called dicer enzyme, and expression in cells. Each method has unique features and benefits appropriate for different scientific approaches. Chemical synthesis, for example, facilitates research that requires a large amount of defined siRNA, while in vitro transcription methods are ideal for screening siRNA sequences or short-term studies. Digestion of long dsRNA produces a heterogeneous population of siRNAs but does not require the design and testing of specific siRNA sequences before hand. Expression systems allow researchers to insert antibiotic resistance markers that help them to select transfected cells.

KITS AND REAGENTS

Companies such as Ambion, **Epicentre**, and **Invitrogen** offer kits and reagents that permit scientists to produce their own siRNAs via whichever method they choose. Alternatively, they can buy siRNA for a large number of published genes, including BRCA1, HPRT, and Lamin, from Dharmacon, **Proligo**, Qiagen, **Stratagene**, and other vendors. These suppliers not only offer tested siRNAs; they also synthesize custom siRNA for researchers and provide items for newcomers to the technology.

"We offer a variety of control products and starter kits for people who have limited experience with siRNA. For example, our RNAi control kit offers all reagents needed to set up and optimize an RNAi protocol," says Qiagen's Tian. The company has also extended the reach of siRNA algorithms. "Working in partnership with **Novartis Pharmaceuticals**, we have studied design using a unique algorithm that's different from those primarily based on Tuschl's design rules," Tian continues. "The result is more potent siRNA to target the genes of interest with high specificity."

Ambion has contributed research as well as products. "We were there at the beginning with our rapid uptake kit," Brown says. "And our research effort has allowed us to develop products in-house that permit researchers to focus on the biology. We're the tinkerers in the corner who create the protocols that enable everyone else to do the biology and get important insights into the science." Now, he continues, "We pride ourselves on being a one-stop shop. We have siRNA tools that target every known gene in mouse, human, and rat. Our delivery reagents cover the broadest spectrum of cells on the market. We've also developed transfection based reagents that enable us to get into any cell type."

The company also offers siRNA libraries. Its Silencer Nuclear Hormone Receptor siRNA Library targets 51 human nuclear hormone receptor gene products, and accelerates functional analyses of this class of proteins. "It's very effective in reducing target gene expression," Brown says. "It's useful in validating hits from a screening experiment."

ISOLATION AND PURIFICATION

Traditional procedures for isolating RNA generally focused on larger RNAs, such as transfer, ribosomal, and messenger RNA. Isolating siRNA, by contrast, demands a method that works well with smaller RNAs. Scientists must also ensure that all solutions and materials involved in preparing siRNA are free of the RNase enzyme, which is not a concern when isolating DNA.

Elchrom Scientific has introduced a purification method that gives a yield of typically 60 percent of an incoming full length oligo at 98 percent purity within three hours. "It's a nontoxic hydrogel-based technology that involves electrophoresis followed by a revolutionary elution technology," says Anatoli Tassis, Elchrom's manager of oligo purification. "We've done it so far at a research scale," says Roux dit Buisson. "But we're working with a partner to get it up to industrial scale."

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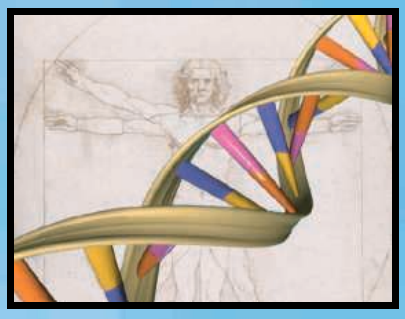

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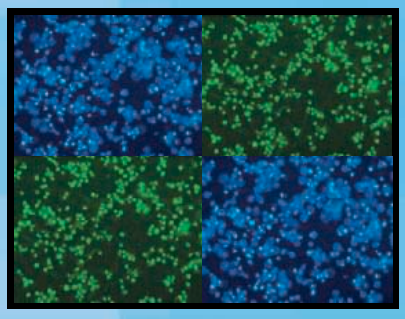

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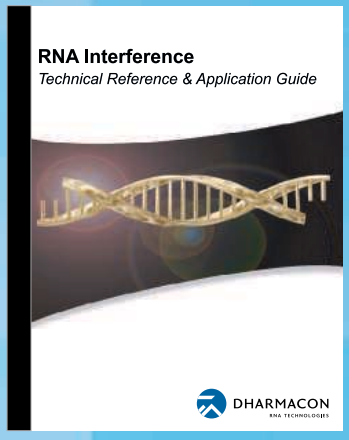

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Detecting and measuring quantities of siRNA presents another problem, because their small sizes prevent their amplification via the favored reverse transcriptase PCR procedure. Most researchers use Northern blots with polyacrylamide gel electrophoresis for this process. Companies such as **GE Healthcare** (formerly Amersham Biosciences) and **Bio-Rad Laboratories** offer instruments and reagents to detect and quantitate RNA and other biological molecules.

MEASUREMENT AND TRANSFECTION

NanoDrop Technologies offers a unique spectrophotometer to measure very small sample volumes by eliminating the need for cuvettes. It can measure concentrations of RNA as low as two nanograms per microliter using a single microliter of sample. "The design is based on the principle of surface tension, which retains the one-microliter sample in place between two optical fibers," Hansen explains. "So you have a direct contact between the sample and the optics without any external sample capture mechanism. With its shorter path length of 0.2 millimeters, the spectrophotometer permits accurate measurement of samples 50 times more concentrated than conventional methods." Since the path length does not depend on the exact volume, the instrument offers excellent accuracy while conserving precious samples.

Once purified, siRNA must be inserted into a living cell to examine its effects on the cell. Some transfection methods accommodate specific cell types; for example, scientists can transfect a plant cell by literally shooting siRNA from a gun through its rigid cell wall. More sensitive cells require more delicate treatment. **Amaxa** and **Gene Therapy Systems** offer such methods as electroporation, viral-mediated transfection, and chemical transfection with cationic liposomal and polyamine based agents for mammalian cells. Mirus Bio, which specializes in technologies for delivering and labeling genes, has developed the first broad spectrum, siRNA-specific transfection reagent, which it calls TransIT-TKO. This reagent facilitates highly efficient siRNA transfection with significantly less cell damage than that caused by cationic-liposome based transfection reagents. "We developed it so that scientists could use modest amounts of siRNA, which is costly, and have the ability to knock out the specific gene they want to," Hagstrom says. The company's most recent product, the TransIT-siQUEST transfection reagent, complements TKO by boosting the efficiency of knockdowns for which TKO is not maximally effective. "Our goal is to attain the highest knockdown efficiencies – in the 80 or 90 percent range," Hagstrom says.

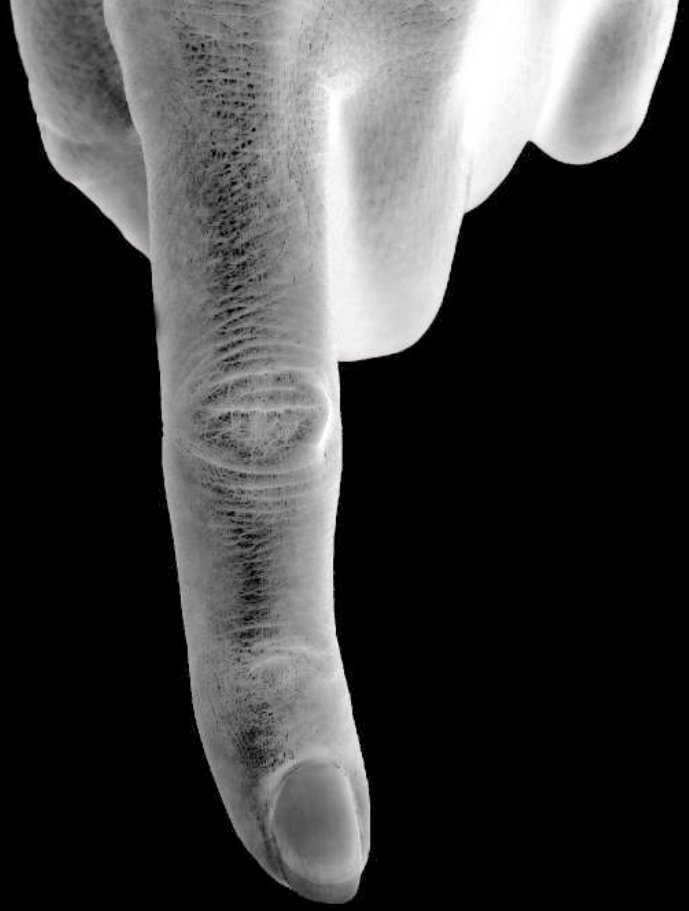
VARIETY OF APPLICATIONS

Gene silencing mediated by siRNA has a wide variety of applications. Prominent examples include research on cell signaling, drug discovery and validation, and human therapeutics.

Upstate offers siRNA/siAb assay kits for studying genes involved in cell signaling. The kits supply what is necessary to knock down a targeted message and determine the level of the knockdown. The kits resulted from a collaboration with Dharmacon. "Dharmacon contributes the siRNA design and chemistry and we contribute the antibody and cell lysate components," Herzig says.

Each kit guarantees the knockdown of the targeted mRNA by at least 75 percent within 24 hours and allows scientists to detect the targeted protein in a Western blot. Dharmacon designed its reagents to make the siRNA more effective in its ability to knock down the targeted message and to reduce the chances of any off-target effects mediated by siRNA. "Our reagents have a high reliability to function in assays," Marshall says. "We want to make sure that anyone who does an RNAi experiment with an interesting outcome will be able to correlate the knocked down gene with the outcome."

Cenix Bioscience, meanwhile, has developed expertise with RNAi-based screens that can be carried out in several model organisms, including *C. elegans* and *Drosophila* cells, to identify new disease-relevant genes. "By doing this we can start looking at the idea of RNAi-based phenotypic profiling of genes that we call phenoprinting," Echeverri says. "That means that we can go through small, medium, and large collections of genes, up to an entire genome, and identify therapeutically relevant genes. We can also use the approach to determine how drugs work, by combining drug treatments with RNAi screens." The company's specialization in the high throughput application of RNAi allows it to save time and reduce costs in carrying out even the most complex and large-scale, cell based validation projects. "Most of our work now is in cultured cells," Echeverri says. "But we look forward to doing some in vivo in animals, although obviously not as high throughput."



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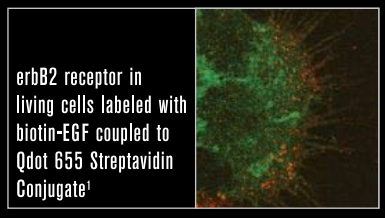

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1. Lidke et al. (2004) Nature Biotechnology, 22(2).

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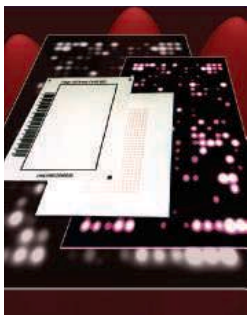

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Scientists can synthesize small interfering RNAs chemically in the lab and then introduce them into cells, thereby opening up great possibilities for using siRNAs as potential drug candidates. Such drugs have several potential advantages over small molecules. For example, they can target virtually any protein without the class restrictions common to some small molecule drugs. They can also simplify the process of drug discovery, as they may not require the extensive lead optimization seen with small molecule drugs. Pharmaceutical companies, including **Acuity Pharmaceuticals** and **Sirna Therapeutics**, are also betting on siRNA for their therapeutic programs.

Two years ago Alnylam, named for the bright star in the center of Orion's belt, became the world's first company focused on RNAi therapeutics. "The major focus of our internal R&D program is to impart chemical modifications to RNAs to give them desirable attributes like increased chemical stability – siRNAs being fragile molecules that don't last long in the bloodstream," Mahanthappa explains. "We have explored chemical modifications that improve siRNA's stability and will increase the probability that the cells we want to treat will see the molecule and turn the gene off." The company is also working on covalent modifications of siRNA to increase its half-life while circulating in the bloodstream and to increase its cellular uptake.

Mahanthappa sees two broad categories of disease that RNAi could treat. "We have systemic RNAi in which we'll inject the siRNA into the bloodstream," he says. "And there's direct RNAi in which we'll administer siRNA directly into the region of the body affected by the disease – such as the appropriate part of the brain for Parkinson's disease."

AGGRESSIVE THERAPY

The most promising therapeutic application of the technology relies on the direct approach. "We aim to inhibit the growth of new blood vessels in the retina that cause macular degeneration," Mahanthappa explains. "We'll inject siRNA into the vitreous humor and expect it to find its way to the retina." Alnylam has taken an aggressive approach to the therapy. "It's our goal to be in the clinic with macular degeneration by 2005," Mahanthappa asserts.

For the alterative approach, he continues, "The liver is a great locus for systemic treatments. We think that therapeutic possibilities involve the regulation and treatment of various blood disorders and hepatitis B and C." Other conditions that could benefit from RNAi therapy include metabolic diseases such as diabetes, high cholesterol, and obesity; viral diseases; autoimmune diseases; and cancer.

The relative simplicity of using siRNA for gene silencing has persuaded many researchers to start using this new research tool. Suppliers have developed a significant offering of tested siRNAs and siRNA libraries, as well as kits and reagents for research use. And biopharmaceutical companies are exploring the tool for therapeutic use. RNA interference has generated more excitement and activity in a shorter time than most advances in research technology. Scientists will soon see what potential this new tool can bring to genomics, cell biology, and medicine.

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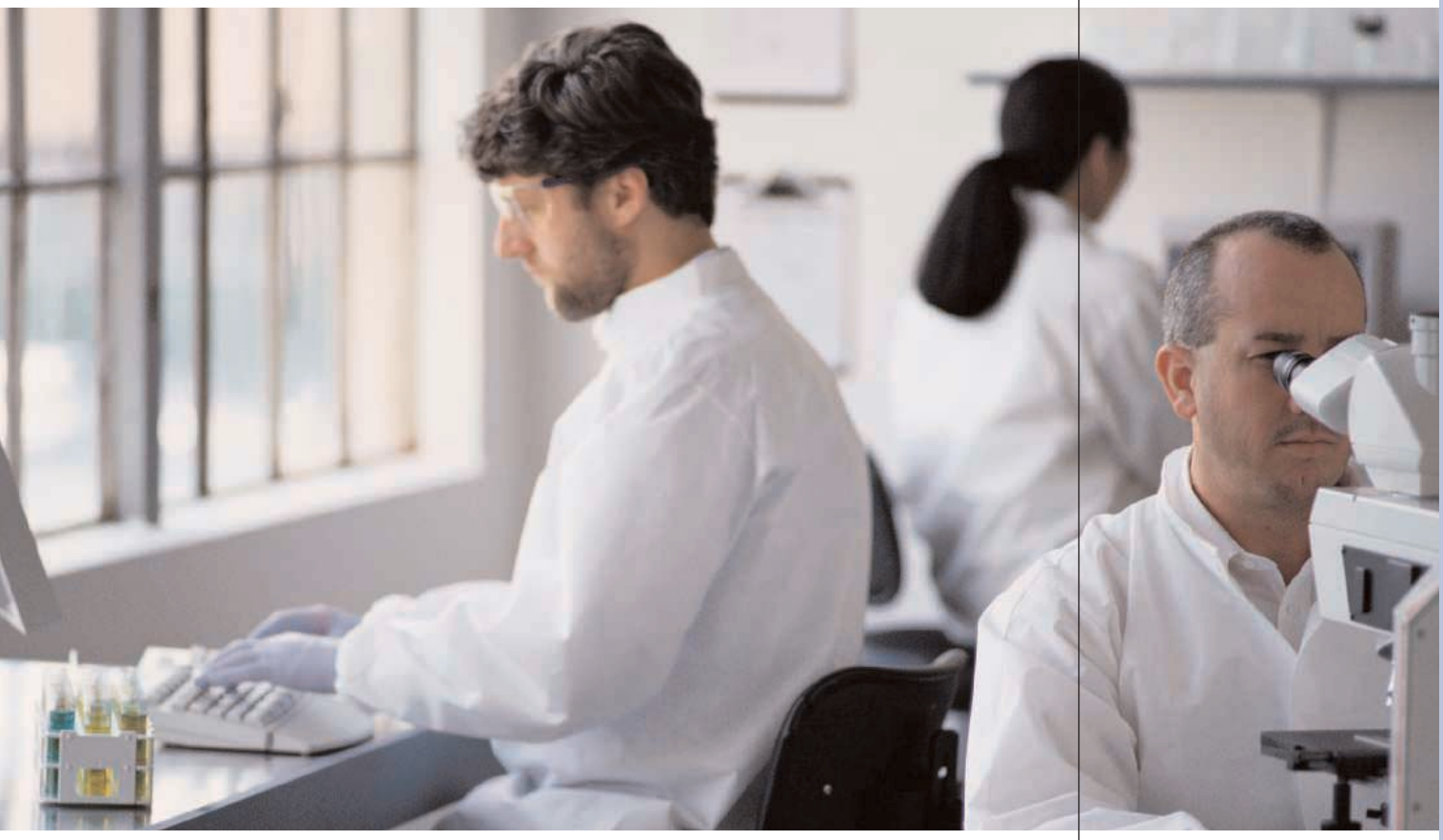
Success Factors for Postdocs



Ensuring a Fruitful Fellowship

A survey sponsored by *Science* Careers reveals the factors that past and present postdocs regard as most critical to the success of their fellowships. BY PETER GWYNNE

What factors have the most influence on the success of a postdoctoral fellowship? In an online survey sponsored by *Science* Careers, more than 900 present and past postdocs, the majority of them in life science departments, ranked the importance of characteristics of principal investigators (PIs), including mentoring skills, publishing records, and the laboratory environment that the PIs create, in determining the success of the postdoctoral experience. Survey respondents also rated institutions responsible for postdocs on such factors as their ability to fund research and their commitment to career fairs and general job guidance.

The survey asked participants to rate the significance of a dozen specific characteristics of their principal investigators or supervisors. Of those, respondents generally assigned the most significance to five factors: mentoring (which 83 percent of participants viewed as very important or extremely important); a sense of direction and vision (81 percent); the ability to obtain funding and grants for postdoctoral work (79 percent); encouraging postdoctoral fellows to carry out and publish research that appeals to potential employers (78 percent); and helping postdocs to network with the scientific community (75 percent).

Success Factors for Postdocs

The PI's Influence

Respondents agree that a postdoctoral fellow's principal investigator or supervisor has far more influence on the success of the fellowship than the institution. That's hardly surprising. Fewer than half of the survey's participants indicated that the institution played a significant role in their choice of location for their fellowship. Respondents predominantly see the selection of their PI as the fundamental factor in the success of their fellowship.

Those conclusions confirm anecdotal findings by organizations that focus on the concerns of postdocs. "They are very much consistent with what we've been hearing from our members and the postdoctoral community," says Alyson Reed, executive director of the National Postdoctoral Association. "In particular, the mentoring theme is one that has paramount importance."

Supervisors of postdocs take an even stronger view of the importance of choosing the right PI. Some assert that the choice has many aspects of the selection of a marriage partner. "The postdoc-supervisor relationship has to be viewed as a lifelong relationship rather than one that lasts a few years," says David Eisenberg, investigator of the Howard Hughes Medical Institute at the University of California, Los Angeles. "I make this explicit when a new fellow joins the lab. I say: 'We are linked for the rest of my life. I accept your career as being largely my responsibility. The corollary is that you have to work productively in the crew to make discoveries, write effective papers, meet people, and communicate well.' As a supervisor I can warn them of problems in the way they want to go."

The choice of institution obviously has some impact on a postdoctoral experience. Academic organizations, in particular, have recently started to realize that they have an obligation to help school their postdoctoral fellows. "For years universities didn't pay attention to postdocs at all, even though they are the next generation of professors," says Kevin Campbell, an investigator for the Howard Hughes Medical Institute and professor and interim head of physiology and biophysics at The University of Iowa. "But there's a lot that universities can do to better support their postdocs, such as giving them opportunities to teach and helping them with long-term career plans."

This supplement will detail the methodology and results of the survey. It will feature comments from survey takers, on an anonymous basis. And, it will include the observations of individual PIs whom survey participants nominated as being among the best in ensuring effective fellowship experiences for their postdocs.

Methodology and Results

Science Careers surveyed members of the American Association for the Advancement of Science, which publishes this magazine, and readers of *Science*. Because other organizations have recently carried out their own studies of postdocs, the team limited the number of potential participants to 10,000 Ph.D.s in the United States who were participating in or had completed postdoctoral fellowships. An online invitation to participate issued in March 2004 resulted in 932 completed surveys, a significant proportion for this type of endeavor.

Wherever they received their training, respondents to the survey had similar expectations for their time as a postdoctoral fellow. The large majority expected their experience to include learning new research techniques, expanding their skill sets, publishing research papers, and ultimately establishing a permanent scientific career.

One survey taker defined the expectations as follows: "A successful postdoctoral experience would include gaining useful technical and intellectual experience by which to broaden future career opportunities. This is achieved by interaction with a PI who is dedicated to both quality research and developing the postdoctoral candidate for future career opportunities. Interaction with fellow postdocs and scientists is also an essential component of a successful postdoc experience." Another postdoc put it rather more philosophically: "A successful experience must be one that fosters independence, provides a strong foundation in the area of study, and promotes a continuing relationship with the postdoctoral mentor." Another individual summarized the situation succinctly: "The postdoctoral experience should be viewed as an apprenticeship with the purpose of gaining scientific, technical, and other skills that advance the postdoc's professional career."

Current postdocs focused more on the activities with which they were involved at the time of the survey, while individuals who had completed their fellowships took a longer view, emphasizing to a greater extent the need to establish a career path and to develop networks and relationships with scientific colleagues. Scientists who had completed their fellowships also had more realistic understanding of career opportunities available to postdocs. While 58 percent of current postdocs want to obtain tenure-track academic positions, only a quarter of former postdocs had managed to land that type of job. And while only 18 percent of present postdocs expect to find jobs in biotechnology or pharmaceutical companies, 31 percent of former fellows actually work for biopharmaceutical firms.

Success Factors for Postdocs

Tier One

At least 75 percent of survey participants regarded the group of factors in this tier as very or extremely important.

- Mentoring
- Direction and Vision
- Funding/Grants
- Employer/Situation
- Networking

Tier Two

Between 55 percent and 66 percent of survey participants considered the group of factors in this tier as very or extremely important.

- Advancement Opportunities/ Career Options
- Work Culture/Environment
- Compensation and Benefits
- Quality of Life
- Training
- Communication

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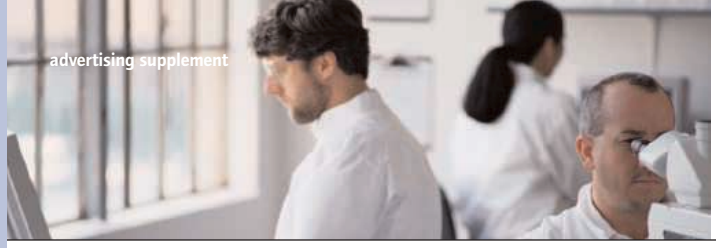

Success Factors for Postdocs

Top Principal Investigators by Success Factor

PRINCIPAL INVESTIGATOR	DEPARTMENT	INSTITUTION	CITY, STATE
Mentoring			
Joseph Bonventre*	Harvard-MIT Division of Health Sciences and Technology and Department of Medicine	Brigham and Women's Hospital, Harvard Medical School and MIT	Boston, MA
Kathryn Holmes	Department of Microbiology	University of Colorado Health Sciences Center	Denver, CO
Richard Lyttle*	Women's Health & Bone Discovery	Wyeth Research	Collegeville, PA
Direction and Vision			
Kevin Campbell*	Departments of Physiology and Biophysics; Neurology	University of Iowa College of Medicine	Iowa City, IA
Richard Dixon	Plant Biology Division	Samuel Roberts Noble Foundation	Ardmore, OK
Juli Wade	Departments of Psychology and Zoology; Program in Neuroscience	Michigan State University	East Lansing, MI
Funding/Grants			
John Cunningham*	Department of Hematology/Oncology	St. Jude Children's Research Hospital	Memphis, TN
Adrian Whitty*	Drug Discovery	Biogen Idec	Cambridge, MA
Allen Wilcox	Epidemiology Branch	National Institute of Environmental Health Sciences	Durham, NC
Employer/Situation			
Ashutosh Chilkoti	Department of Biomedical Engineering	Duke University	Durham, NC
David Eisenberg*	HHMI, Departments of Chemistry & Biochemistry and Biological Chemistry	University of California at Los Angeles (UCLA)	Los Angeles, CA
Matthew Mescher	Center for Immunology (and Dept. of Laboratory Medicine & Pathology)	University of Minnesota	Minneapolis, MN
Networking			
Karlyne Reilly*	Mouse Cancer Genetics Program, Center for Cancer Research	National Cancer Institute	Frederick, MD
John Tamkun	Department of Molecular, Cell and Developmental Biology	University of California, Santa Cruz	Santa Cruz, CA
Watt Webb*	School of Applied and Engineering Physics	Cornell University	Ithaca, NY

Note: Individuals listed in the above table are representative of those with top scores in each category and are listed by alphabetical order. An asterisk indicates individuals interviewed for this article.

Success Factors

What factors play the strongest roles in determining the success of fellowships as defined by the past and present postdocs? Organizers of the survey provided a choice of 12 specific characteristics controlled by the fellow's principal investigator and five determined to some extent by the institution that hosts the postdoc. Respondents were asked to rate those characteristics' contributions to successful fellowships on a sliding scale.

Respondents' rankings of the dozen factors under the control of PIs fitted neatly into three separate tiers. The first tier contains five characteristics that at least three quarters of the respondents consider very important or extremely important to their success as postdoctoral fellows: mentoring, direction and vision, funding and grants, assistance in finding employment, and networking. The second tier, which 55 percent to 66 percent of respondents rate as very important or extremely important, consists of advancement opportunities and career options; the work and culture environment of the PI's laboratory; compensation and benefits; quality of life; training; and communication between PI and postdoc. The final tier contains a single factor: Just 34 percent of the sample ranked the influence of a postdoc's spouse or partner as very important or extremely important. That doesn't come as a surprise; many respondents do not have spouses or partners who might influence their decisions.

The institution plays a much smaller role in postdocs' criteria for the success of their fellowships. Even the highest rated factor controlled by the institution – funding and grants, viewed as very important or extremely important by just 63 percent of survey takers – would not have reached the top tier of characteristics determined by principal investigators. Of the other institutionally influenced factors, compensation and benefits garnered a 48 percent ranking as very important or extremely important, quality of life 44 percent, networking 36 percent, and spouses or partners just 28 percent.

Other factors that participants in the survey regarded as important to successful postdoctoral experiences include transitioning into a permanent career, networking and collaborating with other scientists, and teamwork and other interpersonal skills. Individuals also point out the

continued >>

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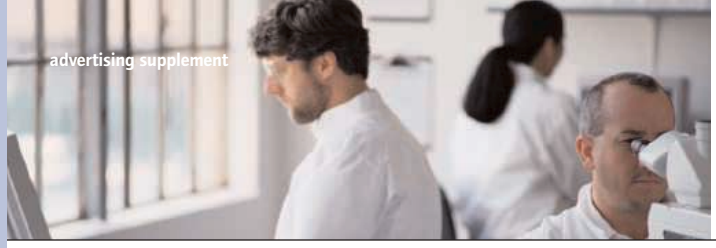
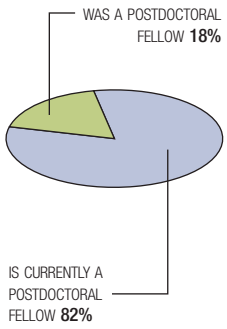


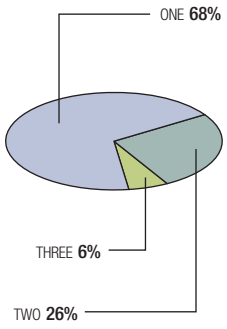
Success Factors for Postdocs

Demographics of Survey Participants

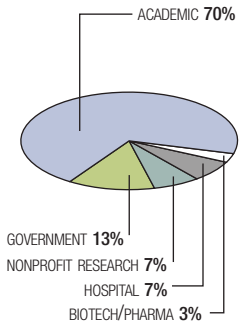
Postdoc Status of Survey Participants



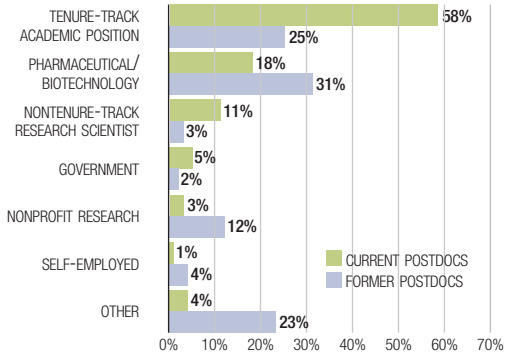
Number of Postdoc Positions Held



Type of Postdoc Institution Rated



Positions Desired by Current Postdocs/ Positions Obtained by Former Postdocs



necessity of doing one's homework – that is, learning as much as possible about the PI and the institution – before accepting any fellowship.

The Meaning of Mentoring

If postdocs had to choose a single characteristic of a principal investigator that will ensure the success of a fellowship, the survey says that they would almost unanimously select mentoring. "Mentoring is key to a successful postdoc experience," one respondent wrote. "Guidance/ mentoring from a PI regarding career options and planning for a career after my postdoc should be required. When postdocs enter a lab, they should design a plan for what they hope to accomplish during their postdoc. Periodically, this plan should be evaluated by the postdoc and mentor to make sure progress is being made."

Principal investigators mentioned as particularly effective mentors by survey participants agree. "Mentoring is probably the most important factor in ensuring a successful postdoc experience," says C. Richard Lyttle, vice president of discovery in the Women's Health Research Institute and co-chair of the women's health and bone therapeutic area at pharmaceutical company Wyeth. "Doing a postdoc means moving from being a student to becoming an independent investigator. Mentors are there to help fellows grow up and go out to compete."

While highly regarded mentors have a variety of styles, they agree on the need for communication. "I try to talk with my fellows on an individual basis twice a week in the lab," says Joseph Bonventre, a director of the Harvard-MIT Division of Health Sciences and Technology (HST), and director of the renal division at Brigham and Women's Hospital in Boston. Lyttle recalls that he had a regularly scheduled weekly meeting with his most recent postdoctoral fellow. "But he knew that he could see me at any time," he adds. "Most of my people are able to find their way," says Watt Webb, professor of physics at Cornell University. "But when I see that they're not, I step in."

Effective mentoring involves two major strands. "A lot of mentoring is done by example. The PI sets the tone for the personal interactions in the laboratory," Bonventre explains. "But it's also important to monitor what postdocs are doing and to avoid overlaps between the work of different postdocs as much as possible." Bonventre also encourages his postdocs to learn how to write papers and grant applications and to develop the management skills necessary to run their own laboratories. Webb, meanwhile, aims to help his postdocs to work comfortably in an interdisciplinary environment. "Mentoring in my lab is done largely by collaboration across disciplines," he says.

Direction and Vision

The second most important characteristic of PIs – providing a sense of direction and vision for postdoctoral research – is a somewhat ambivalent concept. "It is essential that postdocs work in a group in which they have a defined project that will lead to significant publications," explains Campbell of the University of Iowa. "But the contradiction is that real research is not well defined. You need to give each postdoc a vision that encourages them to think of novel and creative experiments."

Adrian Whitty, head of quantitative biochemistry at biopharmaceutical firm Biogen Idec, takes a similar view. "It is very important that the group has a clear vision of what it is trying to achieve and what kind of questions are or are not relevant to these broad scientific objectives," he says. "However, this vision should not be so constraining that it deprives the postdoc of any flexibility when selecting the specific questions to be studied and the approaches to be used. Only in this way does the postdoc have an opportunity to gain firsthand experience at making such judgments, which to my mind is a key component of comprehensive postdoctoral training."

Effective PIs must walk a narrow path between too much and too little autonomy for their fellows. "Postdocs often have better ideas of what to do," says Eisenberg of UCLA. "I as a supervisor can warn them of problems in the way they want to go." Whitty adds "I feel strongly that a fairly high level of autonomy is key to maximizing the value of the postdoctoral experience. Within broad bounds set by the mentor, the postdoc should be free to make strategic and tactical decisions concerning the direction of his or her work." However, he points out, that approach involves a tradeoff. "By allowing the postdoc more say in the direction of the project, even at the cost of making the occasional mistake or false

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Immunobiology and Cancer Program

Assistant Member. A full time faculty-level appointment is available in the Immunobiology and Cancer Program of The Oklahoma Medical Research Foundation. We seek a highly interactive individual to complement and enhance the Program strengths in lymphocyte development and activation. Applicants will have a Ph.D. or M.D. degree, a demonstrated commitment to academic excellence, and the capacity to develop a well-funded research program. Desired areas of specialization could include developmental biology of the immune system, lymphoid malignancies or immune deficiency diseases. Interested individuals should include a C.V., a summary of current research, and names of individuals for reference. Please send inquiries to: Dr. Paul W. Kincade, c/o Kerry Humphrey, OMRF Immunobiology & Cancer Program, 825 NE 13th. St., Oklahoma City, OK, 73104. For information about the program consult www.omrf.org/imb.

K. Mark Coggeshall, Ph.D.
Immunobiology & Cancer

Gary J. Gorbsky, Ph.D.
Molecular, Cell and Developmental Biology

Paul Kincade, Ph.D.
Immunobiology and Cancer

James B. Rand, Ph.D.
Molecular, Cell and Developmental Biology

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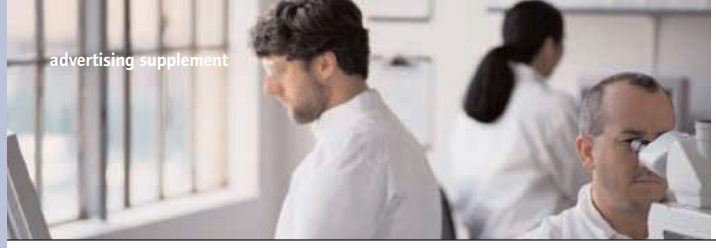

start," he says, "the work may advance more slowly." Eisenberg points out a further key factor in any lab's or scientist's vision of research. "There has to be flexibility about what the goal is," he says. "It can change."

Funding and Grants

Survey participants also pinpoint the ready availability of funding and grants for their research as a key success factor. Here again, successful PIs agree. Most aim to fund their postdoctoral research fully, while encouraging the fellows to search for ancillary grants.

"Our postdocs have no pressure obtaining research grants. I say that whatever experiments they want to do, we can support financially. After all, a postdoctoral fellowship is still an educational process," says John Cunningham, an associate member in hematology-oncology at St. Jude Children's Research Hospital in Memphis. "But I encourage my postdocs to apply for other grants. Learning how to do so is important for their careers." Campbell at The University of Iowa has a similar attitude. "The major funding in the laboratory is the PI's responsibility," he says. "I want to keep the postdoc free of that pressure, but I expect all of my postdocs to pursue their own fellowship funding even though I have the funding for their research activities. This is an important part of their postdoctoral training; writing individual grant applications is part of the learning process for becoming a successful, independent professor."

Principal investigators usually accept the need to prepare their postdoctoral fellows for careers, in academe, government, or industry, and thereby fulfill the demands of respondents to the survey. "You can have all the good mentoring in the world," says Wyeth's Lyttle, "but how you are judged ultimately is based pretty much on your resume. It's your ticket to get in there." The company lists cutting edge science and the opportunity to publish in good journals as key criteria in determining the need for postdoctoral positions. And it aims to include its postdocs as the first authors on papers that it submits to journals.

Working up a research project into a publishable paper is a skill that most postdocs must learn. Here again the PI should take a leading role in the instruction. "Success in research depends critically on communication," UCLA's Eisenberg says. "Learning to write and speak is essential. We need to have a culture in which the communication is continuous."

Using Connections

Helping a postdoc to take the first career step involves more than encouraging publications. PIs routinely use their connections to help place their fellows. "You encourage people you know in the field to go to postdocs' presentations," Lyttle says. "And you need to find out where your postdocs are best suited to work, whether in the pharmaceutical business or in academe."

Equally important, and recognized by participants in our survey, is giving postdocs the opportunity to network within the scientific community, by making presentations, attending meetings, and getting together informally with specialists in their fields. "Often, individuals are recruited for a job interview because of a personal contact – the people in charge of hiring have met the individual or have seen their work at a meeting, and have felt it fit the program's goals," says Karlyne Reilly, a principal investigator at the National Cancer Institute. "Because there's an element of luck to finding the right job, the more you can increase your luck by getting to know people in your field."

Reilly encourages her fellows to present a poster at at least one national meeting a year and to attend as many local meetings as possible. "There they can meet people with different ideas from the way we think about our science within our lab," she says. "They should also get practice defending their ideas, an important factor for their future work. And when I'm meeting colleagues, I try to have postdocs working on relevant research come with me."

Webb at Cornell also encourages his fellows to network at meetings and through personal introductions. "It's crucial that they should start thinking about it more than a year before they start looking for a job," he says. "Early on, it's really networking to communicate in science; the job business comes later."

Bonventre of Brigham and Women's Hospital and HST wants to expose his postdocs to the entire range of networking opportunities. "No matter what they are going to do," he says, "it's important to have networking across the academic-industry barrier. You'll tend to focus more on one direction, but try to expose fellows to the other side."

Institutional Contributions

While institutions feature as relatively minor contributors to postdoctoral success in the survey, authorities have recently started to increase their support for fellows. The National Cancer Institute provides dedicated funds to send fellows to meetings. "We also have local faculty meetings to which we're encouraged to bring our postdocs," Reilly says. "And when we have speakers visit, we set up lunches for them with our postdocs." At St. Jude, adds Cunningham, "We have a five-year-old Academic Programs department that recruits postdocs and provides support throughout their time here." Biogen Idec also has a formal setup for dealing with its fellows. "The director of our postdoctoral program meets individually with each postdoc on a quarterly basis, to discuss whether the program is meeting their expectations," Whitty says.

Other organizations are adapting their programs to the growing needs of fellows. "We're looking at our whole postdoc program," says Wyeth's Lyttle. "We have a postdoc day when the postdocs meet with senior staff here and present their work. And we often try to set up external collaborations with academics. That lets the postdocs see what goes on in the academic side." Universities increasingly recognize the need to provide more institutional support for postdoctoral fellows. "They can make it easier for them to apply for grants, give special workshops on applying for fellowships, and take away some of the administrative load that comes with grants," Iowa's Campbell says.

The National Postdoctoral Association offers its own encouragement to postdocs and their mentors. "We're developing toolkits that postdoctoral associations and offices can use to bring about institutional changes," Reed says. "We're working through institutional and postdoctoral leadership to create opportunities for PIs to focus on issues of importance to postdocs to a greater extent than in the past. And, we're particularly interested in the bigger picture as seen by former postdocs. We're trying to find ways to connect the wisdom of former postdocs to present postdocs."

A former science editor of Newsweek, *Peter Gwynne (pgwynne767@aol.com) covers science and technology from his base on Cape Cod, Massachusetts, U.S.A.*

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Postdoctoral Opportunities

Mammalian Cell Culture Development
(Ph.D. in Molecular/Cellular Biology or related discipline)
- Study of CHO cell biology utilizing gene chip and molecular biology techniques to improve cell culture processes. **Req. #1000004062**
- Engineering of CHO cell lines to manipulate glycosylation profiles. **Req. #1000004063**

Protein Engineering
(Ph.D. in Biochemistry or related discipline)
- Investigate biochemical aspects of protein/protein interactions and structure function relationships. Collaborative multidisciplinary projects in protease enzymology, growth factors/receptors, molecular recognition. Background in molecular biology, proteins, structure, phage display. **Req. #1000003757**

Pharmaceutical Development and Technology
(Ph.D. in Chemical Engineering or related discipline)
- Evaluate technologies for analyzing protein aggregation, including light scattering, field flow fractionation and analytical ultracentrifugation. **Req. #1000004075**
- Focus on mathematical modeling and laboratory characterization of transport phenomena in biopharmaceutical processing (e.g. lyophilization, freezing and thawing of protein solution). **Req. #1000004076**

Analytical Chemistry Development
(Ph.D. in Analytical Chemistry or related discipline)
- Develop capillary electrophoresis (CE) based methods for nucleic acid analysis. **Req. #1000004060**
- Develop qualitative and quantitative mass spectrometric methods for profiling glycosylation and other heterogeneity of natural and recombinant antibodies. **Req. #1000004061**

Molecular Oncology
(Ph.D. in Molecular Oncology or related discipline or M.D.)
- Study the mechanism of regulation of angiogenesis, with an emphasis on tumor angiogenesis. Apply genomic and proteomic approaches to identify novel angiogenic regulators and utilize state-of-the-art tools to elucidate the in vivo and in vitro functions of these molecules. **Req. #1000002928**

Immunology
(Ph.D. in Immunology or related discipline or M.D.)
- Extensive research experience in immunology and molecular biology with good publication record, to study T cell differentiation and development, cytokine function, and the roles of T cell subtypes in autoimmune diseases. **Req. #1000001009**

Molecular Biology
(Ph.D. in Immunology or related discipline or M.D.)
- Explore and define the function of recently discovered cytokines at the molecular level. Make use of animal models and sophisticated in vitro techniques. Applicants should have a strong immunology background. **Req. #1000001513**

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POSTDOCTORAL and **JUNIOR RESEARCH-TRACK POSITIONS** are available for highly motivated career-oriented individuals to: (1) study the functions of estrogen receptor coregulators and upstream growth-factor signaling components as related to hormone-resistant, non-genomic, and chromatin remodeling functions; (2) characterize transgenic mice expressing MTA1 family members and PELP1/MNAR mutants; (3) Characterize knockout mouse models of MTA1 and PELP.

(For recent publications from the laboratory, see: *NCB* **3:**30, 2001; *Nature* **418:**654, 2002; *NCB* **4:**681, 2002; *Cell* **113:**142, 2003. *J. Biol. Chem.* **276:**38272, 2001, 2002; *EMBO J.* **21:**5437, 2002; *J. Cell Biol.* **161:**583, 2003.)

Candidates should have a Ph.D. in molecular biology, cell biology, or genetics. Candidates with prior publications and direct experience will be preferred. Salary is highly competitive and commensurate with experience.

For immediate consideration, send curriculum vitae to: **Rakesh Kumar, Professor and John G. and Marie Stella Kenedy Memorial Foundation Chair, The University of Texas M. D. Anderson Cancer Center–108, 1515 Holcombe Boulevard, Houston, TX 77030. E-mail: rkumar@mdanderson.org.**

The University of Texas M. D. Anderson Cancer Center is an Equal Opportunity Employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability, or veteran status, except where such distinction is required by law. All positions at M. D. Anderson are considered security sensitive; drug screening and thorough background checks will be conducted. The University of Texas M. D. Anderson Cancer Center values diversity in its broadest sense. Diversity works at M. D. Anderson. Smoke-free environment.

POSTDOCTORAL SCIENTIST
Analytical Biochemistry

The Biosystems Research Department at Sandia National Laboratories in Livermore, California, seeks Postdoctoral Scientists to perform research in bioanalytical chemistry. The individual will work in a multidisciplinary team involving analytical chemists, biochemists, and engineers to develop portable devices and assays for clinical diagnostics. Responsibilities will include developing novel protein modification, labeling, and immobilization chemistries; protein purification and characterization; and developing immunoassays for clinical analytes. Research may also involve working with mammalian cells and bacteria.

Qualifications: Ph.D. in chemistry, biochemistry, or a related field is required. Experience with one or more of the following is necessary: bioconjugate chemistry, immunoassays, enzyme assays, protein separation, protein purification, and cell culture techniques. Experience with spectroscopy, optical microscopy and/or mass spectrometry is desired.

Interested candidates should apply electronically at **website: http://www.sandia.gov.** The job requisition number is 050879.

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POSTDOCTORAL RESEARCH ASSOCIATE
National Jewish Medical and Research Center

Position immediately available in the laboratory of **Dr. Brian J. Day** to help discover and develop novel catalytic antioxidants and apply these novel compounds in acute and chronic lung injury models. Seeking Ph.D. in molecular biology/pharmacology or toxicology with experience in cell culture, molecular biology, biochemistry, and analytical chemistry (high-performance liquid chromatography, gas chromatography/mass spectroscopy). Excellent benefits and competitive salary. Please send curriculum vitae and three references to:

Dr. Brian J. Day
National Jewish Medical and Research Center
1400 Jackson Street, K706
Denver, CO 80206
E-mail: dayb@njc.org

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POSITIONS OPEN

THE CENTER FOR GENOMIC SCIENCES
www.centerforgenomicsciences.org

POSTDOCTORAL POSITION to study the molecular biology of medically important mucosal biofilms, and to explore the clinical relevance of horizontal gene transfer among bacterial strains during chronic biofilm infections. The Center for Genomic Sciences is seeking an energetic person to work in multidisciplinary team environment who is interested in bacterial evolution and genetics. Applicants should have extensive experience in all aspects of bacterial strain construction with preference given to those with experience in *H. influenzae* or *S. pneumoniae*. Position is NIH-grant supported. Send letter of interest, curriculum vitae, and copies of manuscripts to **e-mail: gehrlich@wpahs.org.**

POSTDOCTORAL POSITIONS in molecular/cell biology of bacterial pathogenesis. Postdoctoral positions are available in the laboratory of **Dr. Randall Holmes** at the University of Colorado Health Sciences Center. Potential projects include: investigating structure, function, and trafficking of cholera toxin from *V. cholerae*, and the related heat-labile enterotoxins from *E. coli*; characterizing the closely related metalloregulatory proteins DtxR and IdeR from *C. diphtheriae* and *M. tuberculosis* and their roles in global regulation of iron-dependent gene expression and virulence; and developing novel structure-based methods for treatment and prevention of diseases caused by these bacterial pathogens. Research facilities and professional environment are excellent, and we will occupy entirely new research laboratories later this year. Recreational facilities of the beautiful Rocky Mountains are easily accessible. Salary is commensurate with training and experience. Submit curriculum vitae, bibliography, and names of three professional references to**: Dr. Randall Holmes at the Department of Microbiology, Campus Box B-175, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262.** *The University of Colorado Health Sciences Center is committed to Equal Employment Opportunity and Affirmative Action. Citizens and permanent residents of the United States may be considered for positions on an NIH-funded training grant, and individuals from underrepresented groups are encouraged to apply.*

POSTDOCTORAL POSITION
Department of Health and Human Services
National Institutes of Health
National Cancer Institute

The Biomolecular Structure Section (BSS) of the Macromolecular Crystallography Laboratory, National Cancer Institute at Frederick is inviting applications for a Postdoctoral position. Training and experience in macromolecular crystallography are essential. Knowledge and skills in protein engineering, expression, purification, and characterization are desirable. Information about the research activities of the BSS can be found at **website: http://mcl1.ncifcrf.gov/ji.html.** Excellent work environment, state-of-the-art facilities, and regular access to the Advanced Photon Source at Argonne National Laboratory will be provided. Please send curriculum vitae, research summary, and contact information of three references to: **Dr. Xinhua Ji** at **e-mail: jix@ncifcrf.gov.** We offer competitive salary and comprehensive health insurance. *DHHS and NIH/NCI are Equal Opportunity Employers.*

A **POSTDOCTORAL POSITION** is available in the laboratory of **Anna Aldovini** at Children's Hospital and Harvard Medical School to support a research program on HIV biology and AIDS vaccine development. Highly motivated applicants must have a Ph.D. degree, strong experience in molecular biology and/or immunology techniques, and good communication skills. Send curriculum vitae with names and addresses of three references to: **Anna Aldovini, Children's Hospital, Enders 861, 300 Longwood Avenue, Boston, MA 02115** or to **e-mail: anna.aldovini@childrens.harvard.edu.**

POSITIONS OPEN

POSTDOCTORAL SCIENTIST
Microfluidics/Microseparations

The Biosystems Research Department at Sandia National Laboratories in Livermore, California, seeks Postdoctoral Scientists to perform research in application of microfluidics/microseparations to chemical and biochemical analysis. The individual will work on a multidisciplinary project involving analytical chemists, polymer chemists, biochemists, and engineers to develop miniaturized devices and techniques for clinical diagnostics and proteomics research. The research will involve work with laboratory-on-a-chip techniques, spectroscopy, microscopy, and mass spectrometry.

Qualifications: Ph.D. in analytical chemistry, analytical biochemistry, or engineering (chemical, mechanical, or biological) is required. As is extensive experience in one or more of the following areas: microfluidic devices or components, theoretical fluid dynamics, microarrays, electrophoresis, chromatography, immunoassays, and enzyme assays. Knowledge of one or more detection technologies such as fluorescence, electrochemical, and mass spectrometry is also desired.

Interested candidates should apply electronically at **website: http://www.sandia.gov**. The job requisition number is 050878.

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POSTDOCTORAL POSITION is available to study transcriptional control of mammalian oogenesis (**Rajkovic** et al., *Science* **305 (5687):**1157–1159, 2004). We have identified several oocyte-specific transcription factors essential in mammalian oogenesis and continue to use genomic approaches to identify novel genes involved in oogenesis. We utilize mouse knockouts to study the function of these novel genes in mammalian oogenesis.

Experience in molecular biology, cellular biology, protein biochemistry, microarray, and/or animal models are desirable.

The position is available as of September 1, 2004. Please forward your curriculum vitae and names, addresses, and telephone numbers of at least three references to **e-mail: rajkovic@bcm.tmc.edu.**

Aleksandar Rajkovic
Baylor College of Medicine
Department of Obstetrics and Gynecology
Houston, TX 77030
E-mail: rajkovic@bcm.tmc.edu
Telephone: 713-798-8410
Fax: 713-798-5833

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Three **POSTDOCTORAL POSITIONS** are available starting January 2005 in the Department of Medical Microbiology and Immunology, School of Medicine, University of California at Davis (UCD) with **Dr. Shirley Luckhart** to study cell signaling of innate immunity during malaria parasite infection in *Anopheles* mosquitoes. Candidates with peer-reviewed publications and Ph.D. background in molecular cell biology, protein analysis, and/or immunology of infectious diseases preferred. Salary commensurate with experience. Send curriculum vitae and names and contact information for three references to: **Dr. Shirley Luckhart, 3146 Tupper Hall, University of California Davis, One Shields Avenue, Davis, CA 95616.** *UCD School of Medicine is an Equal Employment Opportunity/Affirmative Action Employer.*

POSTDOCTORAL/POSTDOCTORAL SCHOLAR

A position is available to work on aspects of the Mdm2-PTEN-p53 oncogene-tumor suppressor network (*Trends in Biological Sciences* **27:**(9)462–466, 2002). Effective communication skills are an absolute requirement. Salary depending on experience. Ph.D. and/or M.D. required. Interested candidates should send a letter stating interests, curriculum vitae, and three references to: **Dr. Lindsey Mayo (e-mail: ldm11@case.edu).** *Applicants must be U.S. residents or have current visa status.*

NIH POSTDOCTORAL POSITION ION CHANNEL BIOPHYSICS

The National Institute of Neurological Disorders and Stroke (NINDS), a major component of the NIH, and the Department of Health and Human Services, is recruiting to fill a Postdoctoral position to study the mechanisms of gating and selectivity of chloride-conducting ion channels in the chloride channel (ClC) family. Our laboratory (**website: http://intra.ninds.nih.gov/Lab.asp?Org_ID=477**) uses several approaches, including fluorescence spectroscopy and electrophysiology, to understand these phenomena, taking advantage of recently solved chloride channel crystal structures to focus our attention on critical protein domains. Projects include using site-directed fluorescence labelling to monitor conformational changes in prokaryotic ClC proteins, as well as electrophysiology of prokaryotic and eukaryotic chloride channels in cells and lipid-bilayer membranes. The laboratory is part of a lively community of ion channel researchers at the NIH. Candidates must have a Ph.D. and/or M.D. and should have a strong background in electrophysiology or membrane protein biophysics. Salary is commensurate with experience. Interested candidates should send curriculum vitae and a statement of research interests to:

Joseph A. Mindell, M.D./Ph.D.
Membrane Transport Biophysics Unit
NIH/National Institute of Neurological Disorders and Stroke
36 Convent Drive, Building 35
Room 3B1014, MSC 3701
Bethesda, MD 20892-3701
Telephone: 301-402-3473
E-mail: mindellj@ninds.nih.gov

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RESEARCH IN GERMANY

The Alexander von Humboldt Foundation's Research Fellowship Program supports scientists of all nationalities and fields as they conduct research in Germany for a period of six to 12 months. Applicants must be under 40 and have a Ph.D. Scientific excellence and the feasibility of the proposal are the primary selection criteria.

U.S. citizens and residents may also apply for: (1) 3 x 3 (Summer) Research Fellowship (three months per year in three consecutive years and visits may be any time of year); (2) Two-year Research Fellowship (24 consecutive months).

Applications are accepted at any time. For information on these and other programs, see **website: http://www.humboldt-foundation.de** or contact the Foundation's U.S. Liaison Office at **e-mail: avh@verizon.net.**

ADVANCED POSTDOCTORAL FELLOWSHIP. Laboratory of **Stephen B. Liggett** is seeking a fellow with potential for subsequent faculty position at the University of Cincinnati College of Medicine. Research expertise in G protein coupled receptor biology, pharmacogenetics, or transgenic mice preferable. Send resume to: **Stephen B. Liggett, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Room G167, P.O. Box 670564, Cincinnati, OH 45267-0564; telephone: 513-558-0484; fax: 513-558-0835; e-mail: stephen.liggett@uc.edu.** *The University of Cincinnati is an Equal Opportunity Employer and encourages the application of women and minorities.*

POSTDOCTORAL POSITION COMPUTATIONAL BIOLOGY Johns Hopkins University

Immediate position available in the Bader laboratory for a Ph.D. trained in computational biology, bioinformatics, or related theoretical/computational fields. Research areas include biological networks, protein-DNA structural biology, and genetic screens. See **website: http://www.jhubiomed.org** and *Science* **302:**1727, 2003. Applications should be sent to **e-mail: joel.bader@jhu.edu** and include curriculum vitae, research interests, and the names of three references with permission to contact them directly.

POSITIONS OPEN

THE CENTER FOR GENOMIC SCIENCES
www.centerforgenomicsciences.org

POSTDOCTORAL POSITION to study the molecular biology of scarless wound healing. The Center for Genomic Sciences is seeking a highly motivated individual to join a dynamic multidisciplinary team committed to identifying and translating fundamental biological findings in wound repair into improved patient care. The successful applicant will work in a highly cooperative basic research environment in superbly equipped laboratories set within a renowned clinical care facility. Applicants should be proficient in differential gene expression, molecular cloning, and related technologies. Position is NIH funded. Send letter of interest, curriculum vitae, and copies of manuscripts to **e-mail: gehrlich@wpahs.org.**

RESEARCH FELLOWSHIPS Arnold Arboretum

The Arnold Arboretum of Harvard University invites applications for one- and two-year **POSTDOCTORAL FELLOWSHIPS** in the areas of plant evolutionary biology, biogeography, plant systematics, plant development, and plant physiology. Priority will be given to candidates whose research makes use of the extensive living (Putnam Fellowships), preserved (Mercer Fellowships), and library botanical collections of the Arboretum and the Harvard University Herbaria. Candidates must be associated with a research scientist or faculty member based at the Arnold Arboretum, Harvard University Herbaria, or Department of Organismic and Evolutionary Biology. Applications should consist of curriculum vitae, research proposal, required support, statement from a Harvard sponsor, and three letters of recommendation. Review of applications will begin on December 1, 2004. Applications should be sent to: **Dr. Robert E. Cook, Director, Arnold Arboretum, 125 Arborway, Jamaica Plain, MA 02130.** *The Arnold Arboretum of Harvard University is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITIONS Baylor College of Medicine Department of Pediatrics and Center for Cell and Gene Therapy

NIH-funded Postdoctoral positions available immediately to investigate novel oncolytic agents and immunotherapy for treatment of solid tumors. Specific focus is on incorporating therapeutic genes into oncolytic herpes simplex virus to enhance its antitumor effect and to boost antitumor immune responses. Applicants with a strong background in molecular biology and immunology is highly desirable. The candidate must have a Ph.D. and/or M.D. degree, and be able to work independently and communicate effectively. If interested, please contact: **Dr Xiaoliu Zhang** at **telephone: 713-798-1256** or send resume and contact information for three references through **e-mail: xzhang@bcm.tmc.edu.**

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POSTDOCTORAL POSITION Department of Pharmacology, Toxicology, and Therapeutics University of Kansas Medical Center

Tumor Molecular Biologist, Ph.D. To study the role of cell cycle, Aurora-A kinases, and centrosomal protein interactions in animal models of estrogen oncogenesis. Strong background in molecular and tumor biology, cell culture, use of bacterial artificial chromosome/cosmid libraries, and co-immunoprecipitation required. Applicants must be proficient in the use of the English language. Please apply online only at **website: http://jobs.kumc.edu.** Search for position # J0201299.

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POSITIONS OPEN

Two NIH-funded **POSTDOCTORAL RESEARCH POSITIONS** are available immediately to study calcium-dependent signaling in fungal pathogens during host infection. The successful candidates will join a highly motivated and committed research team and use a multidisciplinary approach to resolve the role of calcium channels in promoting fungal pathogenesis. One individual will use patch-clamp techniques and molecular biology to perform structure-function studies of a newly identified calcium channel found in the pathogen. This individual should have extensive experience with electrophysiology and molecular biology. A second individual will examine the interactions between the channel and signaling cascades by identifying protein interactors of the channel and using a murine model of fungal infection. A background in signal transduction, yeast genetics, and ion transport would be an asset. Send or e-mail curriculum vitae, research interests, and names of three references to: **Dr. Angela Gelli, Genome and Biomedical Sciences Facility, Department of Medical Pharmacology and Toxicology, University of California, Davis, 451 East Health Sciences Drive, Room 3502, Davis, CA 95616. E-mail: acgelli@ucdavis.edu.**

POSTDOCTORAL POSITION in ecology and evolution of a bird-borne alphavirus. An NIH-funded Postdoctoral position is available immediately to study the population dynamics of Buggy Creek virus, an alphavirus associated with cliff swallows and their cimicid ectoparasites (see: *Proc. R. Soc. Lond. B* **268:** 1833, 2001). The position includes both field- and laboratory-based work and requires travel to and from performance sites in Nebraska, Colorado, Oklahoma, and Munich, Germany. Applicants should have a Ph.D. and background in virology, epidemiology, or phylogenetics. To apply, send curriculum vitae and names and addresses of three references to: **Charles R. Brown, Department of Biological Sciences, University of Tulsa, 600 S. College Avenue, Tulsa, OK 74104; e-mail: charles-brown@utulsa.edu.** *The University of Tulsa is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL FELLOW POSITIONS are available in cell biology, The Hospital for Sick Children Research Institute, to join our interdisciplinary program studying quality control of membrane protein, structure-function relationship, proteomics, and trafficking determinants of cystic fibrosis transmembrane conductance regulator (CFTR), as well as the role of CFTR in inflammation (see: *Nature Struct. Biol.* **5:**180–183, 1998; *J. Cell Biol.* **164:**923, 2004, and **153:**957, 2001). Candidates must hold a Ph.D. and have a strong background in molecular-, cell-biology, and protein biochemistry. Experience in mass spectroscopy, transgenic mice, or electrophysiology is an asset. Submit curriculum vitae, three references, and description of research interest to: **Gergely L. Lukacs** at **e-mail: glukacs@sickkids.ca.**

A **POSTDOCTORAL POSITION** is available in the laboratory of **Dr. David Dooley,** Department of Chemistry and Biochemistry at Montana State University, Bozeman. This position is open immediately for an individual with a Ph.D. in chemistry, biochemistry, or related field. The individual will conduct research on the molecular biology and protein engineering of metalloenzymes and will utilize spectroscopic and kinetic methods to characterize these enzymes. For more information on how to apply, please see the following **website: http://www.montana.edu/cgi-bin/msuinfo/fpview?ctype=p&csn=247-3.** *ADA/Affirmative Action/Equal Opportunity Employer/Veteran's Preference.*

POSTDOCTORAL POSITION for one and a half years to develop a novel method to identify synthetic genetic relations in yeast and animal cells using microarrays. To begin at once. *U.S. citizens and permanent residents.* Contact **e-mail: alan.tartakoff@case.edu. (Pathology, Case Western Reserve University, Cleveland, Ohio).**

The Argonne National Laboratory Named Postdoctoral Fellowship Program

The Director's Office initiated these special postdoctoral fellowships at Argonne National Laboratory, to be awarded internationally on an annual basis to outstanding doctoral scientists and engineers who are at early points in promising careers. The fellowships are named after scientific and technical luminaries who have been associated with the Laboratory and its predecessors, and the University of Chicago, since the 1940's.

Candidates for these fellowships must display superb ability in scientific or engineering research, and must show definite promise of becoming outstanding leaders in the research they pursue; the Laboratory intends to award four such fellowships this coming year. Fellowships are awarded for a two-year term, with a possible renewal for a third year, and carry a stipend of $70,000 per annum with an additional allocation of up to $20,000 per annum for research support and travel.

Applying for an Argonne Named Postdoctoral Fellowship:

Your application requires the following documents which must be sent via e-mail to: **fellowships@anl.gov** by November 12, 2004.

- Letter of Nomination (Recommendation from individual who supports your candidacy for the fellowship.)
- Curriculum Vitae (Include the names of the Nominator and two additional references.)
- Two letters of reference (It is the candidate's responsibility to arrange that the two reference letters be sent to the Laboratory via e-mail prior to the November 12, 2004 deadline.)
- Bibliography of publications
- Bibliography of preprints
- Description of research interests to be pursued at the Laboratory (We encourage applicants to contact Argonne staff in their areas of interest in order to explore possible areas of research.)

All correspondence should be addressed to ANL Named Postdoctoral Fellowship Program. One application is sufficient to be considered for all named fellowships.

For additional details, visit the Argonne web site at **http://www.anl.gov**.

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EYE AND VISION RESEARCH AT HARVARD

THE POSTDOCTORAL PROGRAM "Molecular Bases of Eye Disease," funded by the National Eye Institute, is designed to provide expertise in the most current molecular research techniques within the context of ophthalmic diseases. Thirty faculty mentors, all with appointments or affiliations with the Department of Ophthalmology at Harvard Medical School, are drawn from laboratories of the Massachusetts Eye and Ear Infirmary, the Schepens Eye Research Institute, the Brigham and Women's Hospital, the Massachusetts General Hospital, the Longwood Campus Basic Science Departments, and the Department of Molecular and Cell Biology on the Cambridge campus.

Participants are expected to spend three-to-four years in training. Training involves (i) participation in full-time research to obtain a mastery of molecular techniques in biochemistry, cell biology, genetics, immunology, or neurobiology as applied to ophthalmology and vision; (ii) exposure to ophthalmic problems through a series of didactic courses, clinical grand rounds, seminars, journal clubs, and meetings; and (iii) writing grant proposals to obtain individual support for years two through four.

Positions are available for individuals immediately after the awarding of their terminal degree, and for those who have completed their postdoctoral clinical training. Candidates must have a Ph.D. or M.D. degree, and be citizens or permanent residents of the United States.

Applicants for this program can obtain information on the mentors and their research at the **website: http://www.eri.harvard.edu/training/index.html**. More detailed information on the application process and the program is available through **Jennifer Schleicher, Schepens Eye Research Institute, 20 Staniford Street, Boston, MA 02114; phone: 617-912-7401; fax: 617-912-0117; email: jenn@vision.eri.harvard.edu.**

The Schepens Eye Research Institute is an Equal Employment Opportunity Employer M/F/D/V.

Postdoctoral Program — Protein Homeostasis

Millennnium Pharmaceuticals Inc. is launching a Postdoctoral Fellowship Program in the rapidly advancing area of Protein Homeostasis. Following on the success of the first-in-class proteasome inhibitor, bortezomib (VELCADE®), for the treatment of cancer, the initiation of this program presents a unique opportunity to join one of the industry's leading multidisciplinary research groups in the field of protein homeostasis. We're looking for highly talented and motivated PhDs in all research areas spanning chemistry, biochemistry, molecular, cellular and *in vivo* biology, algorithms and mathematics to participate in this exciting program. Fellows will integrate closely with the Discovery organization and have a real opportunity to learn the science of drug-discovery. They will be encouraged to present their findings at major scientific meetings and will be expected to publish in leading peer-reviewed journals. We are looking for candidates to work in the following broad areas:

- Analysis and modeling of complex networks related to protein homeostasis
- Biochemistry of the 26S proteasome: expression, localization, associated proteins, regulation
- Biochemistry and regulation of the unfolded protein response
- Biochemistry and regulation of ubiquitin and ubiquitin-like protein modification systems
- Molecular genetic studies (*in vitro* and *in vivo*) of the cellular response to VELCADE®

Preferred candidates will have recently received their PhD or have completed 2 to 3 years of postdoctoral studies. Experience in the area of protein homeostasis is an advantage, although candidates from other scientific backgrounds are encouraged to apply. Appointments will be for one year initially with renewal for a second and possibly a third year.

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Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences

Postdoctoral Research Opportunities

Human health and human disease result from three interactive elements: environmental factors, individual susceptibility and age. The mission of the National Institute of Environmental Health Sciences (NIEHS) is to reduce the burden of human illness and dysfunction from environmental causes by understanding each of these elements and how they interrelate. The NIEHS achieves its mission through multidisciplinary biomedical research programs.

Several postdoctoral positions are available immediately at NIEHS/NIH/DHHS to study: 1) the role of estrogen receptors in the lung; 2) the role of eicosanoids in pulmonary immune function; and 3) the biological significance of cytochrome P450-derived eicosanoids in the cardiovascular system. These projects will utilize newly developed knockout and transgenic mice in established models of disease including allergic and non-allergic lung inflammation, lung fibrosis, cardiac ischemia/reperfusion injury, hypertension and atherosclerosis. Applicants should possess a Ph.D. degree in Molecular Biology, Cellular Biology, Biochemistry, Immunology, Pharmacology, or a related field, and have no more than five years of relevant postdoctoral experience. Salary will be commensurate with experience according to NIH guidelines. For details regarding on-going projects in the laboratory and a list of recent publications, please visit:

http://dir.niehs.nih.gov/dirlpp/groups/zeldin/members.htm

For prompt consideration, send or e-mail cover letter, curriculum vitae including list of publications in peer-reviewed journals, and the names/phone numbers of three people who could provide letters of reference to:

Darryl C. Zeldin, M.D.
National Institute of Environmental Health Sciences
111 T.W. Alexander Drive, Bldg. 101, MD: D2-01
Research Triangle Park, NC 27709
zeldin@niehs.nih.gov

NIEHS

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Cancer Immunology Training Program

The Ohio State University Comprehensive Cancer Center invites applications from motivated young scientists for postdoctoral positions in an NCI-sponsored Cancer Immunology Training Program. Applicants must have a PhD and/or MD and be a US citizen or green card holder. Women and minorities are encouraged to apply. The mentors and their expertise are as follows:
Yang Liu (Professor and Program Director): T cell recognition in cancer and autoimmunity; **Clark Anderson (Professor):** Biology of Fc receptor; **Rolf Barth (Professor):** Neutron capture therapy of brain cancer; **John Byrd (Professor):** Antibody therapy of cancer; **Michael A. Caligiuri (Professor):** NK Cell immunobiology and cancer therapy; **William Carson (Associate Professor):** Clinical cancer immunology; **Philip Johnson (Professor):** Gene therapy for HIV and cancer; **Clay Marsh (Professor):** Signal transduction and apoptosis; **Fredika Robertson (Professor):** Inflammation and carcinogenesis; **Susheela Tridandapani (Assistant Professor):** Fc Receptor and antibody therapy; **Christopher Walker (Professor):** T cell response to hepatitis virus C; **Caroline Whitacre (Professor):** Immunobiology of autoimmunity; **Pan Zheng (Associate Professor):** Cancer evasion of host immunity.

Please send an application to: **Dr. Yang Liu, c/o Lynde Shaw, Division of Cancer Immunology, Department of Pathology, Ohio State University Medical Center, 1645 Neil Avenue, 129 Hamilton Hall, Columbus, OH 43210** or e-mail to: **shaw-5@medctr.osu.edu**. Specify the name of the mentor that you are interested in working with.

STONY BROOK POSTDOCTORAL POSITIONS

The Research Foundation of Stony Brook University/SUNY anticipates the following postdoctoral positions being available between October 2004 and May 2005.

• APPLIED MATH AND STATISTICS

Study protein-ligand binding. James Glimm, #WC-R-1614-04-09-S

• BIOCHEMISTRY AND CELL BIOLOGY

Biochemical mechanism of Wnt signaling. Jen-Chih Hsieh, #WC-R-1616-04-09-S

Biosynthesis, folding, degradation of glyco proteins in yeast. William J. Lennarz, #WC-R-1603-04-09-S

Crystallographic studies of ubiquitin-dependent protein degration. Herman Schindelin, #WC-R-1618-04-09-S

Expression, purification, and biophysical characterization of membrane proteins. Smita Mohanty, #WC-R-1625-04-09-S

Glycosylation and cell wall biogenesis in yeast and pathogenic fungi. Neta Dean, #WC-R-1620-04-09-S

Plant cell and molecular biology plant-pathogen interactions. Vitaly Citovsky, #WC-R-1600-04-09-S

Regulation of endocannabinoid (andandamide) expression. Dale Deutsch, #WC-R-1619-04-09-S

Role of novel virulence factors; molecular mechanism of bacterial pathogenesis. Wali Karzai, #WC-R-1629-04-09-S

Structure and function of caveolin-1 and caveolae. Deborah Brown, # WC-R-1637-04-09-S

Yeast chromatin modifying enzymes. Rolf Sternglanz, #WC-R-1624-04-09-S

• BIOMEDICAL ENGINEERING

Biopolymeric hydrogels for delivering cytokines/DNA to enhance wound repair. Weiliam Chen, #WC-R-1611-04-09-S

Cardiovascular biofluid mechanics, computational fluid dynamics, thromboembolism. Danny Bluestein, #WC-R-1634-04-09-S

Identification of molecular, protein, and histological markers of bone adaptation. Stefan Judex, #WC-R-1613-04-09-S

• CHEMISTRY

Bioorganic approaches to enzyme inhibition, tuberculosis drug discovery. Peter Tonge, #WC-R-1617-04-09-S

DNA, surfactant complexes, colloids, fibers, modified fullerenes, and membranes. Ben Chu, #WC-R-1628-04-09-S

Flow-induced polymer crystallization, spinning processes, nanoparticles, biodegradable polymers, and nanocomposites. Ben Hsiao, #WC-R-1627-04-09-S

Membrane structure: enzymatic probes of lipid domains. Nicole Sampson, #WC-R-1632-04-09-S

Vibrational, NMR, and fluorescence spectroscopy of fluorescent proteins. Peter Tonge, #WC-R-1622-04-09-S

• ECOLOGY AND EVOLUTION

Community/ecosystem ecology of biological invasions. Jessica Gurevitch, #WC-R-1607-04-09-S

Genetics and Phylogeography of herbivorous insects and their host plants. Douglas Futuyma, #WC-R-1612-04-09-S

• MINERAL PHYSICS

High pressure and synchrotron X-rays studies. Jiuhua Chen, #WC-R-1623-04-09-S

• MOLECULAR GENETICS

G protein-coupled receptors/morphogenesis f-pathogenic yeast. James Konopka, #HS-R-1633-04-09-S

• NEUROBIOLOGY AND BEHAVIOR

Electrophysiological analysis of spinal cord functions in rodents. L. Mendell, #WC-R-1604-04-09-S

Study of the structure/function of glutamate receptors. L. Wollmuth, #WC-R-1606-04-09-S

Vertebrate development genetics: axis formation and early patterning in zebrafish. H. Sirotkin, #WC-R-1605-04-09-S

• NUCLEAR CHEMISTRY

Nuclear Chemistry/Physics expertise in nuclear reactions, relativistic energies. Roy Lacey, #WC-R-1602-04-09-S

• PATHOLOGY

Interactions of francisella tularensis with cells of innate immunity. Marie Furie, #WC-R-1615-04-09-S

• PHARMACOLOGICAL SCIENCES

Molecular cellular pharmacology; molecular toxicology; structural biology; cell biology; animal pharmacology. Jeffrey E. Pessin, #HS-R-1636-04-09-S

Neuroimmue interactions in the physiological and pathological mammalia central nervous system. Styliani-Anna Tsirka, #HS-R-1635-04-09-S

Study of molecular mechanisms of carcino-genesis. Arthur Grollman, #WC-R-1601-04-09-S

• PHYSICS AND ASTRONOMY

Accelerator-based experimental particle Physics. John Hobbs, #WC-R-1609-04-09-S

Experimental nucleon decay and neutrino particle physics. Clark McGrew, #WC-R-1610-04-09-S

Nuclear and computational astrophysics: neutron starts, supernovae, gamma-ray bursters. James Lattimer, #WC-R-1631-04-09-S

X-Ray diffractive imaging of biological and materials science specimens. Chris Jacobsen, # WC-R-1608-04-09-S

• PSYCHIATRY

Structure/function of non-image-forming visual system. Larry Morin, #HS-R-1630-04-09-S

• PSYCHOLOGY

Data management and analysis, manuscript preparation, supervision of undergraduate RA's. Anne Moyer, #WC-R-1621-04-09-S

AA/EOE

To apply online and for information, see *www.postdocs.stonybrook.edu* or mail résumés to: Murray Lamond, Office of the President, Stony Brook University, Stony Brook, NY 11794-0701.

D. E. Shaw Research and Development

Computational Chemistry

Extraordinarily gifted computational chemists and other computational scientists are sought to join a rapidly growing New York-based research group that is pursuing an ambitious, long-term strategy aimed at fundamentally transforming the process of drug discovery.

Candidates should have world-class credentials in computational chemistry, biology, or physics, or in a relevant area of computer science or applied mathematics, and must have unusually strong research and software engineering skills. Relevant areas of experience might include the computation of protein-ligand binding free energies, molecular dynamics and/or Monte Carlo simulations of biomolecular systems, application of statistical mechanics to biomolecular systems, free energy perturbation methods, and methods for speeding up evaluation of electrostatic energies—but specific knowledge of any of these areas is less critical than exceptional intellectual ability and a demonstrated track record of achievement. Current areas of interest within the group include the prediction of protein structures and binding free energies, structure- and ligand-based drug design, de novo ligand design algorithms, and the development of special-purpose hardware to accelerate computational chemistry simulations.

This research effort is being financed by the D. E. Shaw group, an investment and technology development firm with approximately $8 billion in aggregate capital. The project was initiated by the firm's founder, Dr. David E. Shaw, and operates under his direct scientific leadership.

We are eager to add both senior- and junior-level members to our world-class team, and are prepared to offer above-market compensation to candidates of truly exceptional ability. Please send your CV (including list of publications, thesis topic, and advisor, if applicable) to career51@desrad.deshaw.com.

D. E. Shaw Research and Development, L.L.C. does not discriminate in employment matters on the basis of race, color, religion, gender, national origin, age, military service eligibility, veteran status, sexual orientation, marital status, disability, or any other protected class.

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UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Postdoctoral Positions at the University of Alabama at Birmingham

The University of Alabama at Birmingham (UAB) is one of the premier research universities in the US with internationally recognized research programs in bone biology and disease, trauma and inflammation research, cancer, diabetes, digestive and kidney diseases, basic mechanism in AIDS pathogenesis, lung disease and Free Radical Biology among others. UAB is committed to the development of outstanding postdoctoral training programs. It is one of the first universities in the US to establish an office of postdoctoral education.

UAB is recruiting candidates for postdoctoral positions for a variety of research projects. UAB faculty are well funded (20th in 2003 NIH funds nationally), utilize multidisciplinary approaches, and provide excellent research training environments that can lead exceptional candidates to entry level positions in academia, government or the private sector. Full medical coverage (single or family), competitive salaries/stipends, sick leave, vacation, and maternity/paternity leave are offered with every position. Depending on the source of funding, other benefits are available. Birmingham is a mid-size city centrally located in the southeast near beaches and mountains and enjoys a moderate climate for year round outdoor activities and a cost of living rate lower than most metropolitan areas.

If interested visit our web site at **www.postdocs.uab.edu**, under Postdoctoral Opportunities to view posted positions. Alternatively, you may send us your cv and cover letter with four key words for research interests (pdf only) to **yvonnet@uab.edu** and we will post this on our web site so that investigators may contact you. **University of Alabama at Birmingham, Office of Postdoctoral Education, 205-975-7020**.

Minority candidates are encouraged to apply.

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- **Reaching your fullest potential**

INVENTQJAYA was founded by Reveo, Inc. (www.reveo.com), a New York based company practicing a unique business model for "InventQbation" and the Edison Technology Portfolio strategy (ETP). Reveo has over 400 patents and many discoveries in the frontiers of science and technology, spinning-out (InventQbating) new, independent business enterprises.

INVENTQJAYA replicates Reveo's successful business model at the invitation of the Malaysian government to achieve technology sovereignty, consistent with Malaysia's Vision 2020.

INVENTQJAYA is a very well-funded, world class laboratory that has attracted luminaries from around the world with diverse ethnic backgrounds and a mission to make breakthrough advances in the following fields of science and technology:

- Photonics
- Nanotechnology
- Electrochemistry
- Advanced Transportation
- Advanced Materials
- Stereoscopic Imaging

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- **Advanced Transportations** (Mechatronics, Design, Controls)
- **Advanced Materials** (Chemist, Liquid Crystals, Metallurgy, Biomaterials, Semiconductor Materials Processing)
- **Electrochemistry** (Fuel Cells, Water Treatment, Air Purification, Photo-/Bio-electrochemistry)
- **Nanotechnology** (Nano-Characterization, MEMs, Nano-Biology, Nanometrology, Nanolithography, Nanofabrication, Nanomaterials)
- **Stereoscopic Imaging** (Displays, Software, 3D Content Production)
- **Photonics** (Optical Displays and Storage, Photonics, and Optical Communication)
- **Scientific Metrology**

Minimum Qualifications:

- A completed PhD in a field pertinent to one of the areas above
- Demonstrated creativity and leadership
- Proficiency in written and spoken English is essential
- Work location in Cyberjaya, Malaysia.

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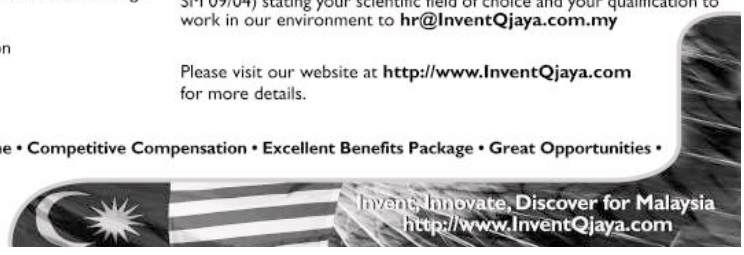

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Endocrine, Reproductive, and Developmental Biology- Postdoctoral Fellow

CIIT Centers for Health Research, Research Triangle Park, NC is seeking a postdoctoral fellow in its Endocrine, Reproductive and Developmental Biology program to study cellular and molecular mechanisms of action of endocrine-active chemicals on reproductive development. A Ph.D. in Endocrinology, Pharmacology, Toxicology, Molecular Biology, Biochemistry, or related discipline is required. Experience in cellular and molecular biology, or biochemistry is preferred.

Interested applicants should send a completed **Postdoctoral Fellowship Application Form** (available from **www.ciit.org**), curriculum vitae, statement of research interests, and three letters of recommendation to:

Human Resources
P.O. Box 12137
Research Triangle Park, NC 27709-2137

Applications may also be submitted electronically to: **Dr. Kevin Gaido**, **919-558-1236** at **gaido@ciit.org**.

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Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes and Digestive and Kidney Diseases

POSTDOCTORAL FELLOWSHIPS IN DNA REPAIR AND RECOMBINATION AT THE NIH

We are a group of molecular and structural biologists whose main interests are in the areas of DNA repair and recombination. We are all located in a single building on the main intramural campus of the NIH in Bethesda, Maryland, a 20-minute ride from Washington, D.C. The intramural program of the NIH offers an outstanding research environment and many opportunities for collaborations. Applications are invited from individuals of the highest caliber with Ph.D., M.D., or M.D., Ph.D. degrees. The current research interests of the group include:

• Biochemistry and molecular biology of double-strand break repair and homologous recombination. Current interests include mouse meiosis (*Mol. Cell* **6:**975, 2000; *Dev Cell* **4:**497, 2003) and evolutionary genomics (*Nat. Genet.* **36:**642, 2004). (**Dan Camerini-Otero**)

• Molecular mechanism of retroviral DNA integration. Biochemical, structural and functional analysis of HIV integrase and other proteins and nucleoprotein complexes involved in retroviral integration (**http://orac.niddk.nih.gov/www/craigie/crahome.html**). (**Bob Craigie: bobc@helix.nih.gov**)

• Structural Biology of Site-Specific DNA Recombination. Current interests include the Adeno-Associated Virus (*Mol. Cell* **10:**327, 2002; **13:**403, 2004), and other eukaryotic and prokaryotic recombination systems. (*Mol. Cell* **5:**1025, 2000; *EMBO J.* **23:**2972, 2004). (**Fred Dyda: Fred.Dyda@nih.gov**)

• Biochemistry and molecular biology of gene rearrangement in the immune system. Current work focuses on the RAG1/2 recombinase, its relation to transposases, and its covalent modification (*EMBO J.* **21:**6625, 2002; *PNAS* **100:**15446, 2003). (**Marty Gellert: gellert@helix.nih.gov**)

• Molecular Mechanisms of DNA repair. Current interests include the role of DNA mismatch repair proteins in homologous recombination and meiosis. (*Nature* **407:**703, 2000; *J. Mol. Biol.* **334:**949, 2003; *Proc. Natl. Acad. Sci.* **100:**14822, 2003). (**Peggy Hsieh: ph52x@nih.gov)**

• Single-molecule biochemical study of macromolecular complex assembly/disassembly dynamics involved in DNA transposition, site-specific recombination and related reactions. (*Mol. Cell* **10:**1367, 2002). (**Kiyoshi Mizuuchi: kmizu@helix.nih.gov**)

• Structural and functional studies of DNA repair (*Mol. Cell* **7:**1, 2001; *EMBO J.* **19:**5962, 2000), and replication (*Nature* **424:**1083, 2003; *Mol. Cell* **13:**751, 2004). (**Wei Yang: wei.yang@nih.gov**)

Interested candidates should send a letter stating their interests, their curriculum vitae and list of publications, and arrange to have letters from three references e-mailed to one of the investigators above or if you would like to be considered for more than one lab to: **Dan Camerini-Otero** (**camerini@ncifcrf.gov**) at: **Bldg. 5, Rm. 201, 5 Memorial Dr., MSC 0538, National Institutes of Health, Bethesda, MD 20892-0538.**

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The Earth Institute
AT COLUMBIA UNIVERSITY

Postdoctoral Fellows Program in Sustainable Development of the Planet

The Earth Institute at Columbia University is the world's leading academic center for the study, teaching, and implementation of sustainable development. It builds on excellence in the core disciplines—earth sciences, biological sciences, engineering sciences, social sciences, and health sciences—and stresses cross-disciplinary approaches to complex problems. Through research, training, and global partnerships, the Institute mobilizes science and technology to advance sustainable development and address environmental degradation, placing special emphasis on the needs of the world's poor.

The Earth Institute seeks applications from innovative postdoctoral candidates interested in path-breaking disciplinary research as well as multidisciplinary initiatives on sustainable development issues. The Postdoctoral Fellows Program in Sustainable Development provides scholars with the opportunity to enhance their foundation in one of the Institute's core disciplines while at the same time acquiring the cross-disciplinary expertise and breadth needed to address critical issues related to reducing poverty, hunger, disease, and environmental degradation.

Candidates for the Postdoctoral Fellows Program should submit a proposal for research based in one of the core disciplines mentioned above that would contribute to the goal of global sustainable development. This could take the form of participating in and contributing to an existing multidisciplinary Earth Institute project, an extension of an existing project, or a new project that connects existing Institute expertise in novel ways. Candidates should identify their desired small multidisciplinary mentoring team, i.e., two or more senior faculty members or research scientists/scholars at Columbia with whom they would like to work during their fellowship. Visit the Institute's Postdoctoral Fellows Web site (http://www.earthinstitute.columbia.edu/postdoc/) for a list of research units and relevant Columbia University and Barnard College departments. Fellowships will ordinarily be granted for a period of 24 months.

More information on the program is available on the Postdoctoral Fellows Web site.

Application forms should be completed online at
http://www.earthinstitute.columbia.edu/postdoc/.

Applications submitted by December 1, 2004, will be considered for fellowships starting in the summer or fall of 2005.

For more information contact:

Hilary Cisneros Dewhurst
Manager, Fellows Program
The Earth Institute at Columbia University
405 Low Library, MC 4335
535 West 116th Street
New York, NY 10027
E-mail: hd6@columbia.edu

Columbia University is an affirmative action/equal opportunity employer. Minorities and women are encouraged to apply.

IRCC
INSTITUTE FOR CANCER RESEARCH AND TREATMENT

Postdoctoral and Research Assistant Positions in Italy

The Institute for Cancer Research and Treatment (IRCC) invites applications for Postdoctoral and Research Assistant positions (tenure track) in the areas of Gene Therapy and Cancer Immunology, Biology of Animal Models, Cancer Genomics, Molecular Imaging, Medical Oncology. Candidates should have a doctoral degree (PhD and/or MD), evidence of outstanding research potential and publication record. IRCC offers competitive salaries according to experience and qualification.

IRCC is an international biomedical research and treatment Centre affiliated with the University of Turin and the Armenise-Harvard Foundation. Its mission is to make a significant contribution to fight cancer by using innovative molecular medicine approaches. IRCC provides training in the area of basic and clinical cancer research and has established successful research programs (***www.ircc.it***). The Institute is located in a lovely environment just outside the city of Turin and very close to the Alps.

Applications should be submitted electronically to the Search Committee at ***recruitment@ircc.it***, indicating the position of interest. Applications should include a CV, a list of publications and the names and contact information for three referees.

POSTDOCTORAL FELLOWSHIP OPPORTUNITIES

The **Santa Fe Institute** (SFI) anticipates several openings for postdoctoral fellowships beginning in September 2005.

SFI research is devoted to complex phenomena drawing input from a wide variety of fields, including biology (e.g., genomics, evolution, ecology, immunology, biochemistry & cellular organization, systems & bioinformatics, structure of non-human social groups), computer science (adaptive & resilient computation, novel forms of computation, simulation), physics and mathematics (nonlinear systems, statistical physics, biophysics), and the sciences of human behavior (cognition, neuropsychological development, cultural evolution, market structure & function, evolution of human language). Applications are also welcome from disciplines other than those listed here.

SFI research is integrative, and there are no formal programs or departments. Postdoctoral Fellows have the opportunity to work either on existing research projects or on projects of their own initiation. Research at the Institute focuses primarily on mathematical and computational approaches, although applicants whose research will include an experimental or data-collection component in collaboration with off-site colleagues are also encouraged to apply. Further details about SFI's current research can be found at <http://www.santafe.edu/indexResearch.php>. Postdoctoral Fellows are appointed for two-year terms on a full-time basis, with the possibility of a one-year extension contingent on funding and performance.

Candidates should have a Ph.D. (or expect to receive one before September 2005), with an academic record of scientific excellence, an ability for independent research, and a strong interest in interdisciplinary approaches and collaboration.

Applications are welcome from candidates in any country. Women and minorities are especially encouraged to apply. Successful foreign applicants must acquire an acceptable visa (usually a J-1) as a condition of employment.

TO APPLY: Please view the full position announcement and application instructions at <http://www.santafe.edu/postdoc05.html>. All application materials must be received via post or electronically submitted no later than **December 1, 2004**. For further information, e-mail postdocinfo@santafe.edu or call (505) 946-2746.

SFI is an equal opportunity employer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
National Cancer Institute

With nation-wide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services (DHHS) oversees the biomedical research programs of the National Institutes of Health (NIH) and those of NIH's research Institutes.

POSTDOCTORAL POSITION TO STUDY THE MECHANISM OF PROTEIN FOLDING USING MULTI-DIMENSIONAL NMR. A post-doctoral fellow position is currently available in DHHS, NIH, National Cancer Institute (NCI), Center for Cancer Research (CCR). The goal of the project is to study the mechanism of protein folding using multi-dimensional NMR. Applicants should have some experiences with NMR and an interest with protein folding, structure, and function. Applicants should have a Ph.D. and fewer than 5 years post-doctoral experiences.

Please send a CV and bibliography, and a list of at least three references to:

Dr. Yawen Bai
Laboratory of Biochemistry
CCR, NCI, NIH, Building 37, Room 6114E
37 Convent Drive
Bethesda, MD 20892-4255 U.S.A.

or by e-mail: yawen@helix.nih.gov

For additional information on the Laboratory of Biochemistry, CCR, NCI please see our website at: **http://ccr.cancer.gov/Staff/Staff.asp?profileid=5607**

DHHS, NIH and NCI are Equal Opportunity Employers.

Department of Health and Human Services
National Institutes of Health
National Institute of General Medical Sciences

The National Institute of General Medical Sciences (NIGMS) in Bethesda, Maryland is seeking applications from outstanding candidates for one Health Scientist Administrator position in the Division of Genetics and Developmental Biology which supports basic, non-disease-oriented research and training.

The incumbent for this position will be responsible for developing and managing a portfolio of research grants that emphasizes genomic and post-genomic approaches to understanding basic biological problems; a portfolio of grants in a related area of genetics, and some of the Division's postdoctoral fellowships. The ideal candidate will have a broad background in genetics and specialized experience in one or more of the following areas: human or mammalian genetics and genomics, proteomics, comparative genomic analysis, and data management and analysis.

Applicants must possess a Ph.D. or M.D. plus scientific knowledge and demonstrated expertise in at least one of the following areas: Biochemistry, microbiology, physiology, genetics, or related areas, and knowledge of the NIH peer review and grants process. Salary is commensurate with qualifications, and includes a full package of benefits. A detailed vacancy announcement (**NIGMS-04-0005**) with the mandatory qualifications and application procedures can be obtained via NIGMS web page at **http://www.nigms.nih.gov/about/job_vacancies.html** and NIH Home page at **http://www.jobs.nih.gov**. Questions on application procedures may be addressed to Erica Greene at (301) 594-2234. Applications must be received by close of business on **October 15, 2004**.

DHHS and NIH are Equal Opportunity Employers

D. E. Shaw Research and Development

Research on Algorithms and Architectures for Computational Biochemistry

Extraordinarily gifted computer scientists, systems architects, electrical engineers and systems software professionals are sought to join a rapidly growing New York–based research group pursuing an ambitious, long-term project aimed at achieving major scientific advances in the field of biochemistry and fundamentally transforming the process of drug discovery. This research effort is being financed by the D. E. Shaw group, an investment and technology development firm with approximately US $8 billion in aggregate capital, and operates under the direct scientific leadership of its founder, Dr. David E. Shaw.

Among the group's current research activities is a project aimed at developing a massively parallel special-purpose supercomputer and innovative mathematical and computational techniques to direct unprecedented computational power toward the solution of key scientific and technical problems in the fields of molecular simulation and molecular design. Successful candidates will be working closely with a number of the world's leading computational chemists and biologists, and will have the opportunity not only to participate in an exciting entrepreneurial venture with considerable economic potential, but to make fundamental contributions within the fields of biology, chemistry and medicine.

Serious candidates will have an exceptionally distinguished history of academic and/or industrial accomplishment in computer science, electrical engineering, applied mathematics, or a related area. Particularly relevant areas of expertise might include parallel computation, high-speed interconnection networks, scientific computing, numerical analysis, optimization, the analysis of algorithms, operating systems, digital systems simulation, reconfigurable computing, and ASIC design, but specific knowledge of any of these areas is less critical than exceptional intellectual ability and a demonstrated track record of achievement. We are prepared to reward exceptionally well-qualified individuals with above-market compensation.

Please send your curriculum vitae (including list of publications, thesis topic, and advisor, if applicable) to career51@desrad.deshaw.com.

D. E. Shaw Research and Development, L.L.C. does not discriminate in employment matters on the basis of race, color, religion, gender, national origin, age, military service eligibility, veteran status, sexual orientation, marital status, disability, or any other protected class.

D E Shaw & Co

POSTDOCTORAL OPPORTUNITIES

POSTDOCTORAL OPPORTUNITIES

The Wadsworth Center of the New York State Department of Health, with basic and applied research programs in the biomedical and environmental sciences, provides a unique and dynamic postdoctoral training experience. Enhancing this environment are state-of-the-art core facilities; broad-based graduate programs with the University at Albany, State University of New York; and new initiatives in bioinformatics, genomics, nanobiotechnology, and biodefense. Positions are available in the following areas:

- **Atmospheric Chemistry**
- **Biodefense**
- **Biomarkers/Nutrition**
- **Cancer Biology/Chemotherapy**
- **Carcinogenesis**
- **Cell Biology/Mitosis**
- **DNA Repair/NMR**
- **Drug Metabolism/Resistance**
- **Gene Expression/Regulation**
- **Immunology**
- **Infectious Disease**
- **Medical Entomology**
- **Microbial Genetics/Pathogenesis**
- **Mobile Genetic Elements**
- **Neuroscience/Disease**
- **Stem Cell Biology**
- **Structural Biology**
- **Toxicology/Neurotoxicology**

For additional information, go to:
www.wadsworth.org/educate/postdocs.htm
and to apply, contact:
Dr. Donal Murphy, Research Office,
Wadsworth Center, New York State Department of Health
P.O. Box 509, Albany, NY 12201-0509
murphy@wadsworth.org

Wadsworth Center

New York State Department of Health
Health Research Incorporated

AA/EOE

FACULTY POSITION

Bioinformatics

Children's Hospital Boston (CHB)
Harvard Medical School (HMS)
and
The Harvard-M.I.T. Division of Health Sciences and Technology (HST)

The Department of Medicine at Children's Hospital Boston and the Harvard-M.I.T. Division of HST at Harvard Medical School seek an outstanding Assistant or Associate Professor in Bioinformatics. Applicants should possess Ph.D., M.D., or M.D./Ph.D. degrees and relevant postdoctoral experience. Areas of particular interest include, but are not limited to: design and application of tools and databases for analysis of genomic sequences, transcriptional profiling data and polygenic contributions to human disease. The successful applicant will enjoy an outstanding competitive startup package, excellent space and state-of-the-art core facilities, and will be able to interact with existing Bioinformatics initiatives at CHB, HMS and MIT. Send CV and description of research and teaching interests, and arrange to have three letters of reference sent by October 15, 2004 to:

Isaac Kohane, M.D., Ph.D.
Director, Children's HST
Informatics Program
Children's Hospital Boston
300 Longwood Avenue
Enders 6
Boston, MA 02115

CHB, HMS and HST are
Equal Opportunity Employers.

POSTDOCTORAL OPPORTUNITIES

LAWRENCE POSTDOCTORAL FELLOWSHIP

The Lawrence Livermore National Laboratory (LLNL) has openings available under its Lawrence Fellowship Program. This is a highly desirable, prestigious postdoctoral position with ample resources and freedom to conduct cutting-edge research in a field of the candidate's choice. The duration of the Fellowship is up to three years. Typically two to four openings are available each year. Fellowships are awarded only to candidates with exceptional talent, credentials, and a track record of research accomplishments.

Candidates will do original research in one or more aspects of science relevant to the mission and goals of LLNL which include: Physics, Applied Mathematics, Computer Science, Chemistry, Material Science, Engineering, Environmental Science, Atmospheric Science, Geology, Energy, Lasers, and Biology. Successful candidates may participate in experimental or theoretical work at LLNL, and will have access to LLNL's extensive computing facilities, specialized laboratory facilities, and field equipment. A senior scientist will serve as a mentor to each of the Fellows. The candidates will receive full management and administrative support. The salary is $7,583/mo.

Please refer to our web page **http://fellowship.llnl.gov** for eligibility requirements and application information. Please reference source code **AJSC9H4DO.** The deadline for application is December 1, 2004. LLNL is operated by the University of California for the National Nuclear Security Administration/Department of Energy. We are an Equal Opportunity Employer with a commitment to workforce diversity.

University of California
Lawrence Livermore
NATIONAL LABORATORY

http://jobs.llnl.gov

Assistant Professor Position
Molecular and Cellular Biology
Harvard University

The Department of Molecular and Cellular Biology (MCB), Harvard University, has several openings for candidates for the position of Assistant Professor.

Our department covers a broad range of topics including cellular biology, developmental biology, molecular biology, molecular evolution, molecular genetics, neurobiology, and structural biology. We are particularly interested in candidates working in the following areas: cellular and molecular approaches to neural circuits, cell biological and evolutionary approaches to development, systems biology and the analysis of genome organization and function, mechanistic analysis of biological action at the molecular and structural levels.

We strongly encourage applications from women and minority candidates. Applications should include: curriculum vitae, reprints of publications, and a statement of present and future research plans (1-3 pages). Complete applications and three letters of recommendation, solicited by the applicant, should be received not later than **19 November 2004**. Mail applications to: **J. Blackbourn/MCB Search Committee, Department of Molecular and Cellular Biology, Harvard University, 7 Divinity Avenue, Rm. 127, Cambridge, MA 02138.**

Harvard University is an Affirmative Action/
Equal Opportunity Employer.

www.mcb.harvard.edu

UNIVERSITY OF CALIFORNIA SAN FRANCISCO

A faculty position in UCSF's Cardiovascular Research Institute (CVRI) is available. The successful candidate will be expected to establish an exciting independent research program and to contribute to CVRI and graduate training programs. Laboratory space and salary support will be provided by the CVRI. A primary academic appointment in an appropriate basic science or clinical department at the **Assistant, Associate,** or **Full Professor** level and membership in UCSF's graduate programs will also be provided. Individuals working in any system (from *C. elegans* to human) in any area broadly related to cardiovascular biology and disease will be considered. Target areas include cellular signaling, molecular imaging at the cell and tissue level, developmental biology, vascular and cardiac biology, genetics/genomics, including human genetics, molecular physiology and disease-focused programs. Outstanding candidates working in other areas will be considered. Candidates for an Assistant Professor appointment should hold a Ph.D. or M.D. and have substantial postdoctoral experience.

By **October 31, 2004**, please submit curriculum vitae, summary of research accomplishments and plans, reprints of major publications, and three to five letters of recommendation to: **Shaun R. Coughlin, M.D., Ph.D., c/o Julie Tom, CVRI, (Search# M-2254), 505 Parnassus Avenue, M1316, University of California, San Francisco, CA 94143-0130**.

The University of California, San Francisco is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.

POSTDOCTORAL OPPORTUNITIES

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH

SALLIE ROSEN KAPLAN FELLOWSHIP FOR WOMEN IN BASIC, CLINICAL, EPIDEMIOLOGICAL OR PREVENTION SCIENCE

The Sallie Rosen Kaplan Fellowship is an opportunity for women postdoctoral scientists in cancer research, made possible by a generous bequest to the Foundation for NIH (FNIH). Candidates for the Kaplan Fellowship must possess a doctoral degree, have less than 5 years postdoctoral research experience, and have U.S. citizenship or U.S. permanent residency (green card). Fellowship training at the NCI can serve as a first postdoctoral assignment, or offer more experienced postdoctoral scientists an opportunity to further their training. The starting date will be no earlier than May 1, 2005 and program duration is normally 2 to 5 years.

NCI's Maryland campuses boast the best funded and equipped research facilities in the United States. Postdoctoral fellows have the opportunity to interact with internationally renowned scientists from a wide range of disciplines. Fellowship stipends are $37,100 to $56,200 commensurate with level of experience. Kaplan Fellow first-year stipends will be augmented with funds generated by the bequest. Standard self and family health insurance is provided and high-option coverage is available.

Applications and supporting letters must be received by December 15, 2004. Applicants are required to apply online. For eligibility information and application instructions for this special opportunity, please go to our training and employment Web site **http://generalemployment.nci.nih.gov** or contact: **NCI Office of Workforce Development, phone: 301-435-8524, fax: (301) 402-3509, e-mail: ncifellows@mail.nih.gov**

DHHS, NIH and NCI are Equal Opportunity Employers

UNIVERSITY OF CALIFORNIA SAN FRANCISCO
Lung Biology

A faculty position in UCSF's Cardiovascular Research Institute (CVRI) is available. The successful candidate will be expected to establish an exciting independent research program and to contribute to CVRI and graduate training programs. Laboratory space and salary support will be provided by the CVRI. A primary academic appointment in an appropriate basic science or clinical department at the **Assistant, Associate,** or **Full Professor** level and membership in UCSF's graduate programs will also be provided. Individuals working in any system (*Drosophila* to human) and any area broadly related to pulmonary biology will be considered. Possible topics include, but are not limited to, invertebrate airway development, mammalian lung development, epithelial cell biology, airway-related innate and adaptive immunity, and the genetics and pathophysiology of pulmonary diseases. Candidates for an Assistant Professor appointment should hold a Ph.D. or M.D. and have substantial postdoctoral experience.

By **October 31, 2004**, please submit curriculum vitae, summary of research accomplishments and plans, reprints of major publications, and three to five letters of recommendation to: **Hal Chapman, M.D., c/o Julie Tom, CVRI, (Search# M2508), 505 Parnassus Avenue, M1316, University of California, San Francisco, CA 94143-0130.**

The University of California, San Francisco is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.

POSTDOCTORAL OPPORTUNITIES

Postdoctoral Positions Available

American Society for Microbiology and National Center for Infectious Diseases

2005 Postdoctoral Research Program

Up to ten associate positions will be awarded by the American Society for Microbiology for full-time research on infectious diseases which cause significant public health problems. Associates will perform research in residence at the National Center for Infectious Diseases (NCID) which is headquartered at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA. In addition to Atlanta, NCID operates laboratories in Ft. Collins, CO, Anchorage, AK, and San Juan, Puerto Rico.

Eligible fields of study include:
Bacterial and Mycotic Diseases
Viral and Rickettsial Infections
Nosocomial Infections
HIV/AIDS
Vector-borne Infectious Diseases
Sexually Transmitted Diseases
Parasitic Diseases

The associate positions are limited to individuals who either earned their doctorate degree (Ph.D., Sc.D., M.D., D.V.M., or D.D.S.) or have completed a primary residency within three years of their proposed start date. The program provides an annual stipend of $35,800, health care benefits package and up to $4,000 for professional development.

The application deadline is **November 15, 2004**. For more information, visit ASM's home page at **http://www.asm.org/Education/index.asp?bid=15497** or e-mail: **Fellowships-CareerInformation @asmusa.org**. The brochure and application are available on line.

American Society for Microbiology

Centers for Disease Control and Prevention
National Center for Infectious Diseases

SCIENTIFIC LEADERSHIP POSITIONS AT ROSWELL PARK CANCER INSTITUTE (RPCI)

As part of an ongoing scientific expansion, RPCI is seeking nationally-recognized leaders in three key research areas described below. During the past five years, RPCI has undergone major changes including the hiring of approximately 100 new senior scientists and clinicians. A new governance structure, initiated in 1999, allows for unprecedented administrative flexibility and competitive compensation and benefits. An expanded academic program offers graduate training to approximately 180 doctoral and masters students at any given time. A $240 million major modernization effort led to a new, state-of-the-art hospital and a 160,000 sq ft medical research complex. In addition, RPCI's new 170,000 sq ft Center for Pharmacology and Genetics will open in January 2006, adjacent to the new University at Buffalo Center for Bioinformatics & Life Sciences and the Hauptman-Woodward Institute Structural Biology Research Center.

At RPCI, basic scientists and clinicians interact in a cancer-focused environment organized into five major research programs supported by an NCI Cancer Center Support Grant (CCSG). Each program emphasizes mission-oriented research culminating in the clinical validation of basic science findings and concepts. In addition to Departmental administration, successful candidates are expected play a leadership role in their respective CCSG program. *All applicants should have substantial and sustained funding.*

CHAIR, MOLECULAR PHARMACOLOGY & THERAPEUTICS

The Therapeutics Department is comprised of 20 scientists and clinicians with interests in target discovery, drug action and resistance and therapy development. Possible areas of applicant research could include, but are not limited to, pharmacogenomics, proteomics, targeted therapies, drug action and resistance, and mechanism-based drug combinations. In addition to developing Department research by recruitment and collaborations, the successful candidate will be expected to coalesce opportunities for target discovery and exploitation being generated by the Genetics faculty and the Center for Bioinformatics, and to expand interactions with the nearby University at Buffalo.

CHAIR, CELLULAR STRESS BIOLOGY

The Cellular Stress Biology Department at RPCI is comprised of 11 scientists and clinicians and focused on radiation therapy, photodynamic therapy and cellular stress responses supported by two P01 awards. Successful candidates will be expected to expand existing programmatic efforts through collaboration and recruitment and to complement clinical research activities by furthering translationally focused research interactions. Areas of research being sought include, but are not limited to, radiation biology, proteomics, photodynamic therapy and oxidative stress biology.

HEAD, DIVISION OF MOLECULAR PATHOLOGY

RPCI is establishing a Division of Molecular Pathology which will support a research interface between clinical and basic science programs. Areas of applicant interest should include biomarker development, gene expression profiling, proteomics or image analysis. The successful candidate will recruit additional faculty in building a strong and collaborative research team responsible for bridging basic and clinical research efforts.

ROSWELL PARK CANCER INSTITUTE

Elm & Carlton Streets
Buffalo, New York 14263
http://www.roswellpark.org
A National Cancer Institute-Designated Comprehensive Cancer Center
A National Comprehensive Cancer Network Member

Each position will be provided with competitive space and financial resources for further development of Departments and Cancer Center Programs. Applicants should submit a CV that includes funding record, three references, and a cover letter indicating the position of interest to: **Michael Brattain, PhD, Senior Vice President, Basic Research, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263, Fax: 716-845-4437, email linda.mckernan@roswellpark.org.**

Roswell Park is an Equal Opportunity Employer

UNIVERSITY OF CALIFORNIA, RIVERSIDE

DEAN

BOURNS COLLEGE OF ENGINEERING

The University of California at Riverside, invites applications and nominations for the position of Dean of the Bourns College of Engineering. UC Riverside offers undergraduate and graduate education to nearly 18,000 students and has a projected enrollment of 21,000 students by 2010. It is the fastest growing and most ethnically diverse campus of the preeminent ten-campus University of California system.

The Bourns College of Engineering is one of the most rapidly expanding engineering schools in the country. It is now on the cusp of a new era for the Riverside Campus. Founded just 15 years ago, the College has 70 faculty and approximately 2,000 students with continued growth expected over the coming years. The College is composed of four departments: Chemical and Environmental Engineering; Computer Science and Engineering; Electrical Engineering; and Mechanical Engineering. The College's physical plant will double in size to 310,000 sq. ft. with the completion of a new engineering building for Computer Sciences and Electrical Engineering. We expect the College programs to grow in size and complexity in the areas of Nanotechnology, Bioengineering and Material Science. More information about the College of Engineering and the University can be found at www.engr.ucr.edu/deansearch.

Major responsibilities of the Dean include academic planning, fiscal management, faculty development, industry relations, and fund raising. Because of the relative youth of the College, program development at both the undergraduate and graduate levels will also be a primary responsibility. The Dean reports to the Executive Vice Chancellor/Provost.

The University seeks energetic, highly motivated and visionary candidates. Essential requirements include a record of distinction in scholarship and the profession, significant management and leadership experience, demonstrated experience in strategic planning, and effectiveness in building collaborative programs across the academic enterprise and with industry and government. The University of California, Riverside, has a strong institutional commitment to the achievement of diversity among its faculty, staff, and students and seeks an individual who shares that commitment.

Salary will be commensurate with experience and qualifications. The starting date is July 1, 2005, or as negotiated.

Review of candidates will begin November 1, 2004; the position will remain open until filled. Applications and nominations should be addressed to:

UNIVERSITY of CALIFORNIA
Riverside

Professor Jerome S. Schultz, Chair
BCOE Dean Search Committee
c/o Sharon Vander Veen
Office of the Executive Vice Chancellor and Provost
University of California, Riverside
Riverside, CA 92506

The University of California, Riverside, is an equal opportunity/affirmative action employer committed to excellence through diversity.

CORNELL THE NEW LIFE SCIENCES INITIATIVE at Cornell University

CORNELL UNIVERSITY HAS ANNOUNCED A $600-MILLION INITIATIVE to recruit faculty and provide resources that foster the multidisciplinary study of organisms in the post-genomics era. This faculty-driven effort serves to integrate the life sciences with Cornell's outstanding programs in the physical, engineering, and computational sciences through 13 interconnected, campus-wide focus areas composing **The New Life Sciences Initiative**. Having begun in 1998 and extending through the next five years, Cornell is in the process of making 110 professorial appointments, establishing a new graduate fellowship program, creating new research core facilities, and building several major capital projects. Active faculty searches are listed on the next three pages. For more information, please visit our websites: http://www.genomics.cornell.edu/, http://lifesciences.cornell.edu/about/initiative.php, and http://vivo.library.cornell.edu.

The main campus of Cornell University, which overlooks 40-mile-long Cayuga Lake, is located in the Finger Lakes region of Upstate New York, a scenic environment of spectacular lakes, waterfalls, gorges, rolling hills, farmland, vineyards, and state parks. It is an area with outstanding recreational and summer and winter sports opportunities for individuals and families. The Cornell campus itself is one of the most beautiful in the country. As a private endowed university that includes several state-assisted colleges and also is the state's federal land-grant institution, Cornell comprises an unusually varied array of academic units. It is a member of the Ivy League. The Ithaca community is culturally diverse with excellent theater, music, sports, and other activities befitting a major university town yet has the warmth and friendliness of a small community. The area is known for its many bookstores and restaurants, an extensive walking trail system, arboretum, ornithology observatory, marina, farmers' market, a hands-on Sciencenter, and art and science museums. For more information and links to individual attractions visit http://www.visitithaca.com/home.cfm.

HOW TO APPLY: Unless otherwise noted, applicants should send a cover letter stating the position(s) for which they wish to be considered, a *curriculum vitae*, a concise statement of research plans and teaching interests, copies of relevant publications, and the names of at least three references to: New Life Sciences Search Committee, Cornell University, 250 Emerson Hall, Ithaca, NY 14853–1901 (email: culifesciences@cornell.edu; phone: 607–254–7261; fax: 607–255–6683). Review of applications will begin November 1, 2004 and continue until the positions are filled. Cornell University is an equal opportunity, affirmative action educator and employer.

Cornell Institute of Molecular and Cell Biology

Cornell University is creating a new Institute of Molecular and Cell Biology (CIMCB) that has as its goal the investigation of fundamental biological processes that lie at the center of life and cellular function. The new institute will serve as a focal point for research efforts being pursued in life science departments across campus, ranging from genetics and neurobiology to plant science, animal science, and veterinary medicine. A key aspect of CIMCB will be strong interdisciplinary interactions. The CIMCB will focus on three key biological processes that occur in a regulatory succession: (1) cellular signaling, (2) the interpretation of these signals at the level of gene regulation and expression, and (3) the execution of this regulation through changes in cellular structure, dynamics, and function.

Schwartz Center for the Performing Arts

While having basic mechanisms of biological function at its core, the intention is that CIMCB will also promote the development of new imaging and other methods to track and manipulate molecules *in vitro* and in living cells, as well as new genetic- and drug-based methods to selectively inactivate or activate particular molecules in specific cells at designated times. The CIMCB will be housed in a new, Richard Meier–designed 270,000-GSF Life Science Technology Building (to be occupied in 2007) where the core institute faculty will be complemented by, and in immediate juxtaposition to, faculty who are developing biophysical, chemical, computational, and engineering approaches that can be brought to bear on high-impact questions in basic biology. Cornell is presently searching for a director. Applications for senior and junior faculty positions in the new institute will be solicited in the near future.

Cell and/or Developmental Biology

The Department of Molecular Biology & Genetics seeks two tenure-track assistant professors of cell biology and/or developmental biology studying the functional organization of cells or tissues. These positions are part of a campus-wide expansion in cell biology (see "Institute of Molecular and Cell Biology" [above]). For one of the positions, there is priority for the use of the mouse as a model organism; this is part of the campus-wide initiative in mammalian genomics. Each successful candidate will be expected to establish a vigorous independent research program and participate in the teaching of cell or developmental biology at both graduate and undergraduate levels. The department includes faculty with research programs in cell biology, developmental biology, genetics, molecular biology, comparative and population genomics, structural biology, and biochemistry (http://www.mbg.cornell.edu). Candidates should submit application materials and arrange for three letters of recommendation to be sent to: Tony Bretscher, Cell/Development Search Committee, 107 Biotechnology Bldg., Cornell University, Ithaca, NY 14853. Applications can also be submitted electronically as PDF files to RLL2@cornell.edu. The committee will begin reviewing applications on October 15, 2004.

Biomedical Engineering

Cornell seeks faculty working at the interface of engineering and life science who thrive on interdisciplinary research and teaching. Cornell has formed a new Department of Biomedical Engineering (BME) that seeks to bridge medicine, biology, and engineering. The department is guided by a vision of developing a quantitative understanding of the human body across scales as a basis for the rational design of devices, diagnostics, and therapies to improve human health. BME is responsible for granting MS/PhD and MEng degrees and sponsors an undergraduate minor in BME available to all students majoring in engineering. Two tenure-track faculty positions in Biomedical Engineering are available with rank open. Research in any area of biomedical engineering will be considered, although there are particular needs for faculty with interests in cellular/tissue bioengineering, molecular/cellular imaging technology, biomaterials, systems biology, and applications of micro/nanofabrication in medicine. Cornell's Center for Materials Research, Nanobiotechnology Center, Center for Nanofabrication, Developmental Resource for Biophysical Imaging and Opto-Electronics, and Theory Center provide outstanding facilities to support interdisciplinary research relevant to biomedical engineering. Candidates should have a PhD in Biomedical Engineering or related technical field. Interested candidates should send a letter of interest, *curriculum vitae*, brief statement of research and teaching interests,

list of recent externally supported research, and names of three references to: Bmep_search-mailbox@cornell.edu.

CORNELL

Biogeochemistry and Biocomplexity

Cornell University invites applications for a tenure-track faculty position in Ecohydrology. We are primarily interested in applicants at the assistant professor level. This individual will investigate and mathematically model the hydrology of ecological systems. Responsibilities for the position include support of the undergraduate and graduate curricula in environmental engineering. Courses taught will reflect her/his areas of expertise and meet departmental needs. Mentoring of undergraduate and graduate students and securing external funding are expected. A PhD with a strong background in quantitative hydrology and/or engineering and the ability to work with ecological systems is required. The candidate must demonstrate proficiency in teaching and have a strong research record. The academic home for this position is expected to be the Department of Biological & Environmental Engineering in the College of Agriculture and Life Sciences. Applicants should submit a letter of application, transcripts, and names of three references (including *curriculum vitae*, telephone numbers, and email addresses) to: Beth Ahner, Search Committee, Biogeochemistry and Biocomplexity Initiative, Biological & Environmental Engineering, 320 Riley-Robb Hall, Cornell University, Ithaca, NY, 14853–5701. We will begin reviewing applications September 15, 2004. For more details: www.bee.cornell.edu/ABOUT/Position.html.

Computational, Statistical, and Evolutionary Genomics

Biological Statistics and Computational Biology is an expanding department that plays a central role in Cornell's New Life Sciences Initiative. With its expertise in Bayesian statistics, bioinformatics, computationally intensive methods, design of experiments, likelihood theory, microarray data analysis, statistical genomics, and survival analysis, this department benefits from a range of exciting interdisciplinary collaborations. We are currently searching to fill one open position at the assistant professor level in the area of bioinformatics, computational genomics, or statistical genomics. Requirements include a PhD in genetics, molecular biology, biochemistry, biostatistics, computer science, mathematics, statistics, or related area with demonstrated research accomplishments in genomics.

Computational Biology and Bioinformatics. The Department of Computer Science is seeking applicants at all ranks for interdisciplinary tenure-track positions in computational biology. Applicants should have a very strong background in computer science and should also have a strong background and research interest in computational aspects of biology. Research may include such topics as development of genomic databases, bioinformatics, biological networks, and structural biology. We are looking for candidates with outstanding research promise who are committed to excellence in teaching computer science. The department is administered by the Office of Computing & Information Science (CIS), a larger unit that can co-sponsor faculty positions in the Faculty of Computing & Information Science with any department on campus. These positions are also part of the campus-wide New Life Sciences Initiative at Cornell which provides ample opportunities for cross-disciplinary research.

Further information about the department and the Office of Computing & Information Science is available at http://www.cs.cornell.edu and http://www.cis.cornell.edu/. Applicants should submit a *curriculum vitae* and the names of at least three references to: Chair, Faculty Recruiting Committee, Department of Computer Science, 4130 Upson Hall, Cornell University, Ithaca, NY 14853–7501.

Human Population Genetics / Epidemiology

Candidates should have expertise in human population genetics, comparative genomics, and/or genetic epidemiology and interests in elucidating the relationships among metabolism, adaptive evolution, genetic diversity, and metabolic disease, and applying this information to understanding the impact of human genetic variation on human metabolism and diet in health and disease. This could include modeling of metabolic pathways if there is emphasis on the role of inter-individual variation. The position is tenure-track at the assistant or associate professor level in the Division of Nutritional Sciences. Successful candidates will be associated with any of several graduate programs, including Genetics & Development, Biochemistry & Molecular Biology, and Computational Biology.


Cayuga Lake Marina

Life Sciences / Physical Sciences Interface

We seek candidates who use innovative approaches to address biological questions or who engineer novel systems based on biological principles. Key areas of interest include, but are not limited to:

The Study of Molecular Events, Interactions, and Dynamics in Living Cells by Advanced and Newly Developed Physical and Chemical Tools. Examples include: the use of state-of-the-art imaging techniques combined with genetic engineering to monitor the dynamics of molecular and genetic networks; the application of spectroscopic and fluorescence methods to monitor protein folding or protein-protein interactions; and the application of chemical synthesis to control or monitor macromolecular reactivity and interactions.

New Approaches to Probing Molecular Structure and Properties. Examples include: the study and manipulation of single molecules by laser tweezers, atomic force microscopy, or electron microscopy; innovative uses of synchrotron radiation to probe structure and dynamics; and the application of protein design to understanding macromolecular interactions.

The Generation of Advanced Materials and Systems Integrating, Mimicking, or Expressing Biological Functionality. Examples include: the development, study, and/or use of new technologies in the areas of microfluidics, polymers, and biomaterials; the design of novel catalysts; and the establishment of new ways to redirect cellular activities by altering enzymatic or transport functions in plants, microbes, or animals.

The Development of New Computational Models and Algorithms to Better Understand Biological Complexity and to Complement and Enhance Experimental Observation. Examples include: understanding the nonlinear dynamics of gene expression and signal transduction, system-wide analyses of transcription and translation, and the use of bioinformatics or statistical mechanics to advance mathematical biology, genomic analysis, and structural biology. All positions are tenure-track at junior and senior levels. Candidates should identify one or two possible home departments in their cover letter.

Mammalian Functional and Comparative Genomics

Cornell is committed to building diverse, campus-wide strengths in mammalian genomics. This long-term initiative involves the recruitment of faculty into several departments, with an emphasis on scientists using genome-wide methods to conduct research in basic and clinical areas of biology. A new Center for Vertebrate Genomics has been established to support research and teaching via mechanisms such as graduate and postdoctoral fellowships.

This year, faculty recruiting in Mammalian Genomics will concentrate on four areas, most prioritizing the use of the mouse as a model organism. However, exceptional applicants using other mammalian or vertebrate systems will be considered. Two of the positions, in **Cell and/or Developmental Biology** and in **Neuroscience**, are detailed elsewhere in this ad. The other two areas are:

Physiological Genomics. The successful candidate will use modern molecular approaches allowing the study of physiological processes on a genome-wide scale. These approaches include, but are not limited to: QTL analysis, gene expression profiling, and proteomics. Ideal applicants will use the mouse, but have a strong interest in extending findings to domestic animals. Contact Yves Boisclair (yrb1@cornell.edu) for more information.

Comparative Genomics. Junior and senior positions are available at the Baker Institute for Animal Health for individuals studying genetic disease and the genomic

organization of domestic mammalian species. Applicants working in the canine system are particularly encouraged to apply. Contact Doug Antczak (dfa1@cornell.edu) for more information.

The positions are available in the departments of Biomedical Sciences, Molecular Biology & Genetics, Animal Science, Molecular Medicine, Clinical Sciences, and the Baker Institute for Animal Health. Candidates should have PhD, MD, and/or DVM degrees, be committed to developing a state-of-the-art, externally funded research program, and be motivated to participate in the university's teaching and public service missions. All positions are tenure-track, available at junior and senior levels, and include competitive start-up packages. To apply, send (preferably as a PDF file) your *curriculum vitae*, statement of research plans, and a cover letter specifying one of the areas listed above to: John Schimenti (jcs92@cornell.edu), Mammalian Genomics Focus Group, College of Veterinary Medicine, Cornell University, T9014A VRT, Ithaca, NY 14853. Also, arrange for three letters of reference to be sent separately (PDFs preferred). General information on Cornell's long-term recruiting objectives in Mammalian Genomics can be obtained through the same contact.

Molecular and Chemical Ecology

Cornell University and the Boyce Thompson Institute for Plant Research (BTI) invite applications for two faculty positions (one junior, one level-open) in Molecular and Chemical Ecology (MaCE). We seek scientists using molecular, chemical, genetic, genomic, biochemical, and/or proteomic approaches to study chemical and genetic bases of interactions between animals, plants, and microbes and/or between organisms and their environment. Research areas include, but are not limited to: molecules that mediate interactions, the receptors and pathways that transduce their signals, and behavioral, developmental, and/or metabolic responses to these molecules. Systems of interest include, but are not limited to, attractive or defensive interactions between animals, plant-microbe interactions, insect interactions with plants or microbes, and natural products chemistry. The successful applicant will be based at BTI or in one of the following Cornell departments: Chemistry & Chemical Biology, Molecular Biology & Genetics, Neurobiology & Behavior, or Plant Pathology. MaCE faculty will join our active, interactive MaCE community, which already spans several Cornell departments and BTI. All MaCE faculty can access multiple genomics and life sciences facilities on campus and are encouraged to form collaborations across the campus. Teaching will be appropriate to the faculty member's expertise and home department. Application materials should be sent directly to: Mariana Wolfner, MaCE Search Committee, Department of Molecular Biology & Genetics, 423 Biotechnology Bldg., Cornell University, Ithaca, NY 14853–2703. Inquiries by email should be sent to mfw5@cornell.edu.

Biological Engineer

The Department of Biological and Environmental Engineering is seeking candidates for a tenure-track position in biological engineering preferably at the assistant professor level. The appointee will establish a research program in an emerging area of biological engineering with nanobiotechnology applications. Research could include molecular recognition and sensing, nanoscale analysis of biomolecules, metabolic engineering, biomimetics, biomaterials, or biosystem-based electronics. Applications of research can be for biological, biomedical, environmental, agricultural, or food systems. The successful candidate will complement ongoing departmental programs in biological engineering activities, particularly at the molecular level, at the Cornell Nanobiotechnology Center. Securing external funding to support research program is expected. Typical teaching responsibilities will be two courses, including one of the core departmental courses, and the development of an upper level undergraduate or graduate course that reflects the candidate's areas of expertise. Mentoring and advising of undergraduate and graduate students is expected. Qualifications: A PhD in an appropriate discipline with demonstrated expertise of integration of engineering and biological sciences. Applicants should submit a letter of application, *curriculum vitae*, transcripts, and names of three references (including telephone numbers and email addresses) to: Michael F. Walter, Department of Biological & Environmental Engineering, 104 Riley-Robb Hall, Cornell University, Ithaca, NY 14853–5701.


Ithaca Farmers' Market

Neuroscience

Cornell University invites applications for three junior faculty positions in neuroscience that use:

(1) genomic/genetic, developmental, molecular, computational, and/or biophysical approaches to the study of excitable cells or tissues (contact David Lin at dml45@cornell.edu for more information);

(2) cell and/or developmental approaches to study the functional organization of the nervous system; for this position, we especially seek individuals with expertise in murine model systems that will contribute to a university-wide interdisciplinary mouse program (contact Tony Bretscher at apb5@cornell.edu for more information); and

(3) integrative approaches to CNS function with interests that could include, but are not limited to, the organization of sensory or motor systems; social behavior, social communication, social cognition; emotion or any other aspect of cognition such as learning and memory at the network level, spatial navigation, or decision-making; a variety of current recording or imaging techniques would be welcome (contact Barbara Finlay at blf2@cornell.edu for more information).

The positions are available in the departments of Biomedical Sciences, Molecular Biology & Genetics, or Psychology. Neuroscience faculty will have access to multiple genomic and life science facilities on campus and are encouraged to form collaborations throughout the campus. Applicants should send a cover letter to: Neuroscience Search, Department of Neurobiology and Behavior, Seeley G. Mudd Hall, Cornell University, Ithaca, NY 14853–2702.

Plant Genomics

Plant Developmental Biology. Applications are sought for the position of assistant or associate professor from candidates whose research focuses on the molecular genetic analysis of plant developmental processes, preferably from an evolutionary perspective. The successful candidate will be based in the Department of Plant Biology and is expected to contribute to teaching in the area of plant developmental biology.

Plant Abiotic or Biotic Interactions

Applications are sought for a tenure-track faculty position (assistant or associate level) from candidates whose research focuses on plant-organismal interactions or responses/signaling related to abiotic stress. Perspectives employing natural variation, genomics, metabolomics, and/or proteomics are preferred. The successful candidate will be based at the Boyce Thompson Institute, an independent not-for-profit institute located on the Cornell University campus. Applicants should submit a *curriculum vitae* and three-page statement of research interests to Maria J. Harrison, Faculty Search Committee, Boyce Thompson Institute, Ithaca, NY 14853. Review of applications will begin November 1, 2004.

Plant Bioinformatics

The USDA-ARS Center for Health-Based Crop Genomics on the Cornell University campus is soliciting applications for a faculty-equivalent scientist using bioinformatic approaches for plant research. The successful candidate will develop bioinformatic tools to integrate and mine genomics, proteomics, and metabolite data related to plant nutritional quality and abiotic stress tolerance. Applicants must have US citizenship and a PhD in an appropriate discipline. Salary range is $69,762 to $82,438 per annum. To obtain application information, visit the USDA web site at http://www.afm.ars.usda.gov/hrd/jobs/index.htm. Applicants are also encouraged to contact Jim Giovannoni (jjg33@cornell.edu) for more information. Applications in response to this ad must include the Vacancy Announcement Number ARS-ARS-X4E-0356. This position will remain open until filled.

Photographs by Cornell University Photography

THE UNIVERSITY OF IOWA

FACULTY POSITIONS AVAILABLE

Presidential Biological Scholar Program

The Carver College of Medicine and the College of Liberal Arts and Sciences at the University of Iowa are seeking new investigators of outstanding promise in the basic biological and clinical sciences. Up to four young investigators will be named as Presidential Biological Scholars at the rank of assistant professor in the tenure-track. The award will provide significant financial research support for a period of four years. Funding for salary and appropriate research space will be provided by a departmental appointment. Scholars may apply directly to the Presidential Biological Scholar Program or be nominated by Department Heads of the Carver College of Medicine's basic and clinical academic departments, or by Department Chairs of units in the College of Liberal Arts and Sciences at the University of Iowa.

Candidates must have a Ph.D., or equivalent, and a demonstrated record of excellence in scholarship as evidenced by publications in leading journals in appropriate disciplines.

Applications, including a cover letter indicating the faculty position of interest, curriculum vitae, list of references, and a summary of research accomplishments and future plans, can be sent to:

Presidential Biological Scholar Program
Richard Smith, M.D., Chair
Attn: Sonya Housholder
Office of the Dean, 200 CMAB
The University of Iowa
Carver College of Medicine
Iowa City, IA 52242-1101

A list of eligible departments is available at:
http://www.uiowa.edu/~pbschol

The University of Iowa is an Equal Opportunity and Affirmative Action Employer. Women and minorities are strongly encouraged to apply.

The Ohio State University

Executive Dean,
Colleges of the Arts and Sciences

The Ohio State University invites nominations and applications for the position of Executive Dean of the Colleges of the Arts and Sciences.

Five colleges constitute the Colleges of the Arts and Sciences: Arts, Biological Sciences, Humanities, Mathematical and Physical Sciences, and Social and Behavioral Sciences. The colleges are home to 41 departments and schools with over 1000 faculty. The colleges provide 60 percent of all credit hours taught at the University, including 90 percent of the General Education Curriculum and 75 percent of all honors credit hours. They offer 71 undergraduate major programs that attract more than 40 percent of the University's undergraduate students. The colleges are linked by a collective mission and share a common commitment to basic research and creative performance and are united by their responsibility for the University's General Education Curriculum (GEC).

The Executive Dean of the Arts and Sciences, who reports to the Executive Vice President and Provost, provides leadership to the five colleges. The position offers the **opportunity to plan the direction and lead the development of world-class programs and initiatives in the arts and sciences in the 21st century; to work collaboratively with the deans of the five arts and sciences colleges in coordinating common programs and services; and to facilitate synergies across the colleges and with other units at the University in research, teaching, and outreach and engagement.** The Executive Dean functions with delegated authority from the Provost. Responsibilities include overseeing the appointment, reappointment and annual evaluations of the college deans, in consultation with the provost; developing college budget recommendations; allocating common funds; and providing administrative oversight of undergraduate student academic services, the honors and scholars programs in the arts and sciences, The Ohio State University Press, interdisciplinary programs in the arts and sciences, development activities, and communications.

The Executive Dean also provides leadership to the University and serves on the Coordinating Council, which provides advice to the Provost on major academic matters; the Council of Deans Steering Committee, which develops the agenda for the Council of Deans; and the University Senate Fiscal Committee, which reviews fiscal policies and budgets.

Qualifications and expectations for the successful candidate include:

- a record of scholarly and teaching achievement appropriate for a senior faculty appointment in one of the disciplines in the Colleges of the Arts and Sciences;
- demonstrated excellence in the leadership and management of large, complex academic programs;
- the ability to serve as an effective advocate and energetic spokesperson for the arts and sciences within the university and externally to students, alumni, donors and funding agencies;
- a history of collaborative working relationships and promoting interdisciplinary partnership;
- a demonstrated commitment to diversity; and
- a record of successful fundraising.

The Executive Dean is appointed to a five-year renewable term, subject to annual reviews by the Provost and a satisfactory formal performance review prior to the end of each term.

The position is available January 1, 2005, or later. Salary and other considerations will be consistent with the University's commitment to recruit the best-qualified individual. To assure full consideration, applications and nominations should be received by October 1, 2004. **The Search Committee will begin screening dossiers on that date and will continue to receive applications until the Dean is selected. Applications should include a statement of interest and a curriculum vitae.** Applications and nominations should be addressed to:

THE OHIO STATE UNIVERSITY

Patrick Osmer, Chair
Search Committee for the Executive Dean
186 University Hall
230 North Oval Mall
Columbus, OH 43210

To build a diverse workforce, Ohio State encourages applications from individuals with disabilities, minorities, veterans, and women. EEO/AA employer.

These are the end products of innovation, drive and a desire to improve the quality of human life.

If the same basic principles and values guide your career, and if you would like to make a difference, we'd like to hear from you. We are Rosetta Inpharmatics, a wholly-owned subsidiary of Merck and Co., Inc. We conduct leading-edge genomic research and data analysis focused on how medical compounds affect biology, enabling more accurate selection of drug targets and more efficient drug development.

We currently offer over 20 exciting career opportunities in the following departments:

Cancer Biology/Gene Modification conducts laboratory experiments directed at developing pioneering applications of RNA interference and DNA microarray technology.

- **Senior Research Biologists**
- **Biologists, RNA Interference Technology**

Informatics researches, develops, integrates and applies cutting-edge methods, databases and software solutions to empower drug discovery and development.

- **Senior Project Manager, Bioinformatics**
- **Senior Research Scientist positions in Custom Analysis, Data Analysis for Gene Expression Technology, Pathway Analysis, and Pathway Bioinformatics**
- **Software Engineering positions in Database Development and Integration, Application Design, and Tool Development and Integration**

Preclinical Molecular Profiling designs and conducts experiments directed at understanding mechanisms of adverse drug responses (toxicity and aberrant drug metabolism) using advanced DNA microarray technology.

- **Senior Investigator/Scientific Director**

Research Genetics develops and applies methods to analyze and mine genetic and molecular profiling data in segregating mouse and human populations to elucidate complex human diseases and identify and validate novel targets for those diseases.

- **Senior Statistical Geneticists**

For more information about our company and our career opportunities, please visit our Web site: **www.rii.com**. To apply for or inquire about any of our open positions, e-mail us at: **rosettahr@merck.com.**

ROSETTA INPHARMATICS
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www.rii.com

Postdoctoral Fellowships

The UNC Lineberger Comprehensive Cancer Center of the University of North Carolina at Chapel Hill will have openings in 2004–2005 in its training program, now in its 29th year, for persons completing graduate studies to train with excellent investigators in basic research in tumor virology, molecular carcinogenesis, molecular therapeutics, cancer cell biology, genetics, tumor immunology and research that interfaces with clinical and physical sciences. Special programs are organized for Fellows including career opportunities in academia and biotechnology.

Training is available in DNA repair, replication and mutagenesis; regulation; regulation of cellular proliferation and differentiation including growth factors, signal transduction pathways and intercellular communication; molecular immunology; tumor virology and pathogenesis; molecular genetics and epidemiology of cancer; and human disease models and gene therapy. Unique training resources and core facilities are supported by the NCI-designated UNC Lineberger Comprehensive Cancer Center.

Preceptors are: Shawn Ahmed, Steven Bachenheimer, Albert Baldwin, Victoria Bautch, James Bear, Keith Burridge, Sharon Campbell, Stephen Chaney, David Clemmons, Edward Collins, Marila Cordeiro-Stone, Blossom Damania, Channing Der, Robert Duronio, Shelton Earp, Beverly Errede, Rosann Farber, Jeffrey Frelinger, Frank French, Jack Griffith, Eng-Shang Huang, Clyde Hutchison III, Kenneth Jacobson, Rudolph Juliano, David Kaufman, William Kaufmann, Shannon Kenney, Ryszard Kole, David Lee, Jason Lieb, Terry Magnuson, Patricia Maness, William Marzluff, Beverly Mitchell, Andrew Morris, Joseph Pagano, Leslie Parise, Mark Peifer, Charles Perou, Tom Petes, Nancy Raab-Traub, James Raleigh, Dale Ramsden, Matthew Redinbo, R. Jude Samulski, Aziz Sancar, Michael Schaller, Jeff Sekelsky, Norman Sharpless, Oliver Smithies, John Sondek, Brian Strahl, Ronald Swanstrom, James Swenberg, Lishan Su, Holden Thorp, David Threadgill, Jenny Ting, Michael Topal, Terry Van Dyke, Kevin Weeks, Bernard Weissman, Elizabeth Wilson, Yue Xiong and Yi Zhang.

Candidates must be U.S. citizens or permanent residents. For an informational brochure or to apply (include curriculum vitae, three letters of recommendation, a statement of research interests and graduate school records) write to: **Joseph S. Pagano, M.D., CB# 7295, UNC Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7295; http://cancer.med.unc.edu.**

UNC's Comprehensive Cancer Center is an Affirmative Action/Equal Opportunity Employer. Minority applicants are encouraged voluntarily to identify themselves.

THE CITY COLLEGE
The City University of New York

FACULTY POSITION IN PHYSICS

The Department of Physics anticipates one or more tenure track faculty positions at the level of Assistant or Associate Professor. One line will be in biophysics. Applicants with expertise in condensed matter physics (including Bose-Einstein condensates or laser physics) are desired for the second position. Candidates should have a Ph.D. degree and postdoctoral experience, a strong record of original research and the ability to establish active, independent, extramurally funded research programs. They must also demonstrate ability and commitment to teaching physics at both the undergraduate and graduate level.

Applicants should submit a CV, a list of publications and a research plan and should arrange for three letters of reference to be sent as soon as possible to: **Faculty Search Committee, Department of Physics, Room J419, The City College of New York, Convent Avenue and 138th Street, New York, NY 10031.** Consideration of applications will begin on October 15, 2004, and continue until the positions are filled.

Additional information available at www.ccny.cuny.edu/positions/.

The City College/CUNY is an EEO/AA/IRCA/ADA Employer.

The University of Michigan
SCHOOL OF DENTISTRY
Chair Position Department of Cariology, Restorative Sciences, and Endodontics

Applications and nominations are solicited for an outstanding leadership opportunity as Professor and Chair of the Department of Cariology, Restorative Sciences, and Endodontics. This will be a full-time, tenured position to lead the largest of six academic departments in the School of Dentistry that comprises the fields of cariology, restorative sciences, and endodontics. The position offers opportunities to further develop interdisciplinary programs at an institution committed to the highest quality of education, excellence in basic, applied and clinical research and service to the public. The department plays an integral role in the delivery of comprehensive patient care, practice management and dental education at the predoctoral and graduate levels. Current research themes are clinical research and cell and molecular biology, and include funded programs in cariology, patient centered research, biomaterials, tooth regeneration, pulp biology and dental public health.

Qualifications include a DDS, PhD, or equivalent doctoral degree and administrative credentials. The successful candidate will have an international reputation as a scholar with outstanding and innovative research accomplishments and an understanding of key aspects of dental education.

Nominations and applications, including curriculum vitae should be submitted to:

David H. Kohn, Ph.D., Chair, CRSE Search Committee
c/o Office of the Dean
School of Dentistry
1011 North University Avenue
University of Michigan, Ann Arbor, MI 48109-1078
Telephone: (734)763-3310

Review of applications will begin no later than November 30, 2004

The University of Michigan is an equal opportunity/affirmative action employer.

House Ear Institute
Advancing Hearing Science

SECTION CHIEF

The House Ear Institute has an opening for an experienced Scientist to develop and direct a research program on the molecular pathogenesis of NF2 (vestibular schwannoma). The House Ear Clinic is a major center for vestibular schwannoma surgery and the House Ear Institute maintains the largest NF2 tumor bank in the world. HEI offers a highly competitive salary and an attractive startup package.

House Ear Institute is affiliated with the University of Southern California. Its scientists have adjunct faculty appointments with USC.

Qualified scientists who are interested in this position should submit a complete curriculum vitae along with a description of past accomplishments in NF or Schwann cell research to:

David J. Lim, M.D., EVP, Research
House Ear Institute
2100 West 3rd Street, 5th Floor
Los Angeles, CA 90057
Fax: 213-483-5675
or e-mail to cyoung@hei.org

Additional information on HEI can be found at **www.hei.org**. EOE.

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

Faculty Position in Brain Tumor Research

An Assistant Member position is available in the Neurobiology and Brain Tumor Program (NBTP) at **St. Jude Children's Research Hospital** (SJCRH). The NBTP is a Cancer Center Program that integrates research groups focused on finding cures for brain tumors. The NBTP has three subdivisions led by Tom Curran (Basic Research), Larry Kun (Clinical Research) and Amar Gajjar (Translational Research). Faculty from several departments studying the basic science and clinical management of brain tumors participate in a collaborative, interdisciplinary research environment. The new Assistant Member would be appointed in an Academic Department appropriate to the applicant's field of research. We are specifically seeking investigators whose research will enhance the translation of basic research findings into the design of new clinical trials for brain tumors. Areas of research interest include, but are not restricted to, the molecular genetic analysis of human glial tumors and the use of animal models to advance understanding and treatment of glial tumors. Applicants should have received their doctoral degree within the past five years and achieved recognition through high profile publications.

SJCRH is the only National Cancer Institute-funded Cancer Center devoted to pediatric cancers. The Hospital operates an active in-house Phase I/II clinical trials program in addition to participating in both the US Pediatric Brain Tumor Consortium and the Children's Oncology Group. SJCRH offers a very competitive package for incoming junior faculty. This includes: salaries for the investigator and their personnel; new laboratory space; as well as startup and continuing support for equipment and consumables. In addition, appointees have access to a range of state-of-the-art institutional core facilities for protein and nucleic acid chemistry, microarray analysis, cell sorting, gene knockout and transgenic technologies and imaging capabilities.

NBTP faculty members with laboratory-based programs focused on brain tumors include:

Tom Curran	Molecular basis of neurodevelopment and medulloblastoma
Suzanne Baker	Normal and neoplastic growth in brain
Michael Dyer	Retinal development and retinoblastoma
Richard Gilbertson	Molecular basis of pediatric brain tumors
Peter McKinnon	Genotoxic stress in the nervous system
Martine Roussel	Cell cycle regulation and brain tumors
Charles J. Sherr	Cell cycle regulation and brain tumors

Candidates interested in the position should arrange to have their CV, a brief research proposal and three letters of recommendation sent to: **Developmental Neurobiology Search Committee, Mail Stop 322, Ms. Wilma Blankenship, St. Jude Children's Research Hospital, 332 N. Lauderdale St., Memphis, TN 38105-2794.**

SJCRH is an equal opportunity employment/affirmative action employer.

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

Faculty Position in Developmental Neurobiology

An Assistant Member position is available in the Department of Developmental Neurobiology at St. Jude Children's Research Hospital (SJCRH). We are specifically seeking applicants with research programs that utilize high-resolution optical imaging in living cells to investigate contemporary issues in neurobiology. Areas of research interest include, but are not restricted to, intracellular macromolecule interactions and trafficking, synapse or receptor dynamics, cell migration and axonal guidance in the nervous system. It is anticipated that successful applicants will have received their doctoral degrees within the past five years and have already achieved recognition through high profile publications.

SJCRH offers a very competitive package for incoming junior faculty. This includes: salaries for the investigator and their personnel; new laboratory space; as well as startup and continuing support for equipment and consumables. In addition, appointees have access to a range of institutional core facilities for protein and nucleic acid chemistry, microarray analysis, gene knockout and transgenic technologies and imaging capabilities that include a state-of-the-art multiphoton microscope. The Department is already well equipped for most aspects of anatomy and histology, cell and molecular biology, transgenics, behavioral analysis and electrophysiology.

There are presently seven faculty members in Developmental Neurobiology with the following research interests:

Tom Curran (Chair)	—	Molecular basis of neurodevelopment
Jim Morgan (Co-Chair)	—	Neuronal death and regeneration
Richard Smeyne	—	Models of neurodegenerative disease
Suzanne Baker	—	Normal and neoplastic growth in brain
Jian Zuo	—	Genetics of hearing and vision
Michael Dyer	—	Retinal development and retinoblastoma
Richard Gilbertson	—	Molecular basis of pediatric brain tumors

Those interested in joining this multidisciplinary department should arrange to have their CV, a brief research proposal and three letters of recommendation sent to the Developmental Neurobiology Search Committee, Mail Stop 323, Attention: Ms. Carol Jacks, St. Jude Children's Research Hospital, 332 N. Lauderdale St., Memphis, TN 38105-2794.

SJCRH is an affirmative action/equal opportunity employer

Harvard University
Theoretical and Computational Biology
Faculty Positions

The Faculty of Arts and Sciences at Harvard University is building a broad, interdisciplinary community in theoretical and computational biology. Up to four faculty positions are open in this area. Successful applicants will be expected to establish a vigorous research program complementing existing activities in the Departments of Molecular and Cellular Biology, Organismic and Evolutionary Biology, Statistics, Mathematics, Physics, Chemistry and Chemical Biology, the Division of Engineering and Applied Sciences, and the Bauer Center for Genomics Research. With the opportunity for synergistic appointments, we encourage applicants to suggest other exceptional candidates including present or potential collaborators. Applications from, or nominations of, women and minority candidates are especially encouraged.

We cannot guarantee that applications received after **November 30, 2004,** will be given full consideration. Please send a cover letter, curriculum vitae, a statement of research plans, and names and addresses of three references to:

Computational and Theoretical Biology
Search Committee
Harvard University
c/o Christine Sprovieri
7 Divinity Avenue, Fairchild 113
Cambridge, MA 02138

Harvard is an Affirmative Action/Equal Opportunity Employer.

ASSISTANT, ASSOCIATE, AND FULL PROFESSORS
MOLECULAR, CELLULAR, AND DEVELOPMENTAL BIOLOGY
CENTER FOR GENETICS AND DEVELOPMENT
UNIVERSITY OF CALIFORNIA, DAVIS

The Center for Genetics and Development, University of California, Davis, invites applications for two tenure-track positions at the levels of Assistant, Associate, or Full Professor. The Center will be a physical and intellectual focal point for research in advanced molecular genetics and development across the five sections of the Division of Biological Sciences and in related campus units. The Center will be recruiting new faculty members at the Assistant, Associate, and Full Professor levels over the next several years. These appointments will be made jointly with an academic unit in the biological sciences as is appropriate to the background and interests of the candidates, and appointments will reside in an appropriate academic department to be determined in consultation with the successful candidate. A Ph.D. (or equivalent) is required. Candidates must have an outstanding record of achievement in research and will be expected to develop a strong research program in an area broadly related to genetics and development. We seek outstanding individuals in all areas of molecular, cellular, and developmental biology, and we seek to augment and complement existing strength in chromosome biology and signal transduction. The successful candidate will be expected to participate in the normal teaching responsibilities of the faculty. Applicants should submit (1) a curriculum vitae, (2) a statement of current and proposed research, and (3) arrange to have at least three letters of recommendation sent to: **Dr. Stephen Kowalczykowski, Director, Search Committee, Center for Genetics and Development, University of California, Davis, One Shields Avenue, Davis, CA 95616-8665.**

Closing date: Open until filled, but all application materials, including letters of recommendation, must be received by October 15, 2004 to be assured full consideration.

The University of California is an Equal Opportunity/Affirmative Action Employer with a strong institutional commitment to the development of a climate that supports equality of opportunity and respect for differences.

R. Gaurth Hansen Assistant Professorship
in Biochemistry
Department of Chemistry and Biochemistry
Utah State University

Applications are invited for the position of R. Gaurth Hansen Assistant Professor in Biochemistry in the Department of Chemistry and Biochemistry at Utah State University. Qualified applicants will have a Ph.D. and postdoctoral experience in biochemistry or a related area. The successful applicant is expected to develop a competitive research program in some area of biochemistry, with preference given to areas that complement existing strengths in the department. This position is one of two that were created in honor of the late **Dr. R. Gaurth Hansen**, former Provost and Academic Vice President at Utah State University and are supported by **Dr. William J. Rutter**, co-founder and former Chairman of the Board of Chiron Corporation.

The Department of Chemistry and Biochemistry (**http://www.chem.usu.edu**) offers Ph.D. and M.S. degrees in Chemistry and in Biochemistry and several degrees at the undergraduate level. The Department has a strong record of attracting extramural support. The recent completion of the new Widtsoe building brings the total departmental space to 124,000 square feet. Utah State University is a land-grant institution with an enrollment of approximately 20,000 undergraduate students and 4,000 graduate students and is located in Cache Valley, 90 miles north of Salt Lake City, in the Wasatch Range of the Rocky Mountains (**http://www.usu.edu**).

The Search Committee will begin screening applications on **October 31, 2004**. Applicants for the position should send a complete curriculum vitae, and the names, mailing addresses, phone numbers, and email addresses of five references to: **R. Gaurth Hansen Search Committee, Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, UT 84322.** Further information about the position can be found at **http://www.usu.edu/hr** and **http://www.chem.usu.edu**.

Utah State University is an Equal Opportunity/ Affirmative Action Employer.

The Jackson Laboratory

FACULTY RECRUITING

The Jackson Laboratory, an independent, mammalian genetics research institution, and an NCI-designated Basic Cancer Center has just launched a major research expansion. New faculty will be recruited in the following areas:

- **Neurobiology**
- **Cancer Biology**
- **Reproductive/Developmental Biology**
- **Immunology/Hematology**
- **Metabolic Disease Research**
- **Computational Biology/Bioinformatics**

We are recruiting scientists at all levels who hold a Ph.D., M.D. or D.V.M., have a record of research excellence and have the ability to develop a competitive, independent research program, taking full advantage of the mouse as a research tool.

The Jackson Laboratory offers a unique scientific research opportunity, including excellent collaborative opportunities with our staff of 35 Principal Investigators, unparalleled mouse and genetic resources, outstanding scientific support services, highly successful Postdoctoral and Predoctoral training programs, and a major meeting center, featuring courses and conferences centered around the mouse as a model for human development and disease. For more information, please visit our Web site (www.jax.org).

Applicants for faculty positions should send a curriculum vitae, statement of research interests, and the names of at least three references to: Director's Office, The Jackson Laboratory, 600 Main St., Bar Harbor, Maine 04609, **or email:** dah@jax.org (preferred method of application).

The Jackson Laboratory is an EEO/AA Employer

The Department of Biochemistry and Molecular Biology at the University of Chicago invites applications and nominations for several tenure-track faculty positions. We seek candidates with outstanding programs and/or promise for engaging fundamental problems and basic molecular mechanisms at the interface of the biological and physical sciences and who will value teaching in the departmental program. We are linked to sister programs in physical sciences, bioinformatics and computation, cell biology, basic medical science, the Institute for Biophysical Dynamics and the Argonne National Laboratory. (See **http://bmb.bsd.uchicago.edu/**)

Applications should include a complete curriculum vitae, a list of publications, a summary of past accomplishments and a plan for future research. Applicants should also arrange for three letters of reference. Please submit applications or nominations by **December 1, 2004** to: **Faculty Search Committee, Department of Biochemistry and Molecular Biology, Mailbox #5, 920 East 58th Street, Chicago, IL 60637**.

The University of Chicago is an Affirmative Action/Equal Opportunity Employer.

UNSW
THE UNIVERSITY OF NEW SOUTH WALES

FACULTY OF SCIENCE
School of Biotechnology and Biomolecular Sciences

Professors/Associate Professors and Senior Lecturers/Lecturers

The School is seeking to make five full-time continuing academic appointments, at both senior and junior levels, across a broad spectrum of areas in Biotechnology and Biomolecular Sciences. These are designed to develop new frameworks and approaches to researching molecular cell biology and biotechnology whilst simultaneously building the capacity to exploit this research within the context of basic, biomedical, environmental and industrial applications.

The appointments will be in two broad areas:

APPLIED BIOTECHNOLOGY/ APPLIED CELL CULTURE

MOLECULAR CELL BIOLOGY/ SYSTEMS BIOLOGY

People from EEO groups including suitably qualified women are encouraged to apply. The University reserves the right to fill the positions by invitation or not to fill all of the positions.

Enquiries to Professor Peter Little, Head of School, on telephone (61 2) 9385 2032; facsimile (61 2) 9385 1483, or email: p.little@unsw.edu.au

For an information package, email: seniorads@unsw.edu.au, or telephone: (61 2) 9385 2730; facsimile: (61 2) 9662 2832.

Applications close 20 October 2004.

For full details of these positions see:
http://www.hr.unsw.edu.au/employment/newjobaca.htm

U32813R

The **Biophysics Research Division at the University of Michigan** invites applications for two open positions in Biophysics. Positions will be at a rank appropriate to the candidate's qualifications and may be at the rank of **Assistant, Associate,** or **Full Professor**. Applications in all areas of Biophysics are encouraged, including both molecular and cellular biophysics and both theoretical and experimental methods. Preference will be given to applicants that complement existing strengths in structural biology, spectroscopy, molecular dynamics, and single-molecule studies. Successful applicants will be appointed in the Research Professor track in Biophysics and will receive a tenure-track teaching appointment in the appropriate department (Chemistry, Physics, Biology, or Biological Chemistry).

All candidates should send a curriculum vitae together with a brief description of their research plans and a statement of their teaching interests, a summary of funding history (senior candidates only) and should arrange to have three letters of recommendation (junior candidates only) submitted to: **Professor James Penner-Hahn, Biophysics Research Division, University of Michigan, 930 N. University Avenue, Ann Arbor, MI 48109-1055**. The positions will remain open until filled but preference will be given to applicants who have submitted all requested materials prior to **November 1, 2004**. Electronic applications are preferred (send to **atitus@umich.edu**).

The University of Michigan is supportive of the needs of dual career couples and is a non-discriminatory, Affirmative Action Employer.

New York University

JOINT FACULTY POSITION
Courant Institute of Mathematical Sciences & Department of Biology
Center for Comparative Functional Genomics

As part of a new interdisciplinary initiative at NYU, applicants are invited to apply for a faculty position (rank open) joint between the Courant Institute of Mathematical Sciences and the Department of Biology/Center for Comparative Functional Genomics to begin September 1, 2005, or as negotiated, subject to budgetary and administrative approval. Candidates working at the interface of Biology (genomics, regulatory networks, cell biology) and Scientific Computation (bioinformatics, simulation, data analysis and cellular modeling) are strongly encouraged to apply. Candidates will be expected to have or to develop active, externally funded research programs, and to participate in teaching activities at both the undergraduate and graduate levels.

The Courant Institute is a leading center in pure and applied mathematics and in computer science, with research strengths of relevance to this initiative in ordinary and partial differential equations, numerical analysis, machine learning, computational biology, and probability theory. **The Biology Department** is undergoing rapid growth in Genomics Faculty and with its Center for Functional Comparative Genomics offers an outstanding and dynamic research environment with a strong emphasis on the integration of genomics, developmental genetics and evolution.

An application letter with research statement, curriculum vitae, and three letters of reference should be sent to: **Chair of the Search Committee, Dr. Leslie Greengard, Courant Institute, New York University, 251 Mercer Street, NY, NY 10012**. Selection of initial candidates will begin ***December 1, 2004***, and proceed on a rolling basis thereafter.

NYU is an Equal Opportunity/Affirmative Action Employer.

2005-06 Congressional Science and Engineering Fellowship Program®

PROGRAM: Scientists and engineers spend one year as special assistants on the staffs of Members of Congress or congressional committees, working in legislative areas requiring scientific and technical expertise. The program includes an orientation on congressional and executive branch operations, and a year-long seminar program on issues involving science and public policy. Fellows receive stipends from their sponsoring societies.

PURPOSE: To provide a unique public policy learning experience, to demonstrate the value of science-government interaction and to make practical contributions to the more effective use of scientific and technical knowledge in government.

CRITERIA: A prospective Fellow must be a postdoctoral to mid-career scientist or engineer; demonstrate exceptional competence in some area of science or engineering; be cognizant of and demonstrate sensitivity toward political and social issues; and perhaps most importantly, have a strong interest and some experience in applying personal knowledge toward the solution of societal problems.

SPONSORS: Approximately 30 national professional scientific and engineering societies will sponsor or cosponsor Congressional Fellows in 2005-06. Applicants should apply directly to the appropriate professional society. Applicants may apply to more than one society. Stipends, application procedures, timetables and deadlines vary by society. Persons from underrepresented minority groups and persons with disabilities are encouraged to apply.

American Association for the Advancement of Science
American Association of Colleges of Pharmacy
American Academy of Neurology
American Chemical Society
American Dental Association
American Geological Institute
American Geophysical Union
American Meteorological Society
American Nuclear Society
American Physical Society
American Psychological Association
American Society for Microbiology
American Society of Agronomy
American Society of Civil Engineers
American Society of Mechanical Engineers
American Veterinary Medical Association
American Welding Society
Crop Science Society of America
Federation of Animal Science Societies
Geological Society of America
Institute of Electrical and Electronics Engineers – USA
Institute of Food Technologists
Institute of Navigation
Materials Research Society
Optical Society of America
Society for Research in Child Development
Soil Science Society of America
SPIE-The International Society for Optical Engineering

For more details about the program and a list of the participating societies contact:

AAAS

ADVANCING SCIENCE, SERVING SOCIETY

American Association for the Advancement of Science
Congressional Science and Engineering Fellowship Program
1200 New York Avenue, NW • Washington, DC 20005
Phone: 202.326.6700 • E-mail: fellowships@aaas.org

www.fellowships.aaas.org

SENIOR FELLOW FOR SECURITY AND TECHNOLOGY

The International Institute for Strategic Studies (IISS), London and the Center for Global Security Research, Lawrence Livermore National Laboratory, California, are seeking a Senior Fellow for a research project on the implications for international security of the latest advances in information and computer technologies taking into account the impact on other related areas such as biotechnology.
Of particular interest is the effect of the accelerating rate of change of technology on global security and the balance between the capabilities of nation states and other agencies such as terrorist groups.

Candidates must have an excellent academic record in a relevant field at the doctoral level or equivalent and demonstrate substantial work and research experience. A sophisticated and objective understanding of international affairs is essential, including in particular, a clear understanding of the interaction of technology and international security policy.

The project will last for a minimum of two years. The Senior Fellow will be expected to divide his/her time between London and Livermore, California.

Salary and benefits will be a matter for negotiation depending on experience and capabilities. A start date of no later than 1 February 2005 is required.

A letter of application, full CV and at least three referees should be sent to Dr Gary Samore, Director of Studies, The International Institute for Strategic Studies, 13–15 Arundel Street, London WC2R 3DX, United Kingdom. Electronic submissions are acceptable and should be sent to hannant@iiss.org. The closing date for applications is 15 October 2004.

IISS
www.iiss.org

ASSISTANT PROFESSOR
Department of Cancer Biology

The Department of Cancer Biology at **The University of Texas M. D. Anderson Cancer Center** is seeking applicants for a tenure track faculty research position at the Assistant Professor level.

Qualified candidates should have a Ph.D. or M.D. degree and must be self-motivated, independent scientists capable of establishing competitive research programs with a focus on cancer biology and the tumor microenvironment. Postgraduate training in the general field of cancer biology, cell biology and/or vascular biology is essential.

Salary is competitive, with an excellent compensation and benefits package.

Interested applicants should send a letter and curriculum vitae to the search committee chair: **Alan. J. Schroit, Ph.D., Department of Cancer Biology, 1515 Holcombe Blvd., Box 173, Houston, Texas 77030**.

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER
Making Cancer History®

M. D. Anderson Cancer Center is an EOE employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status, except where such distinction is required by law. All positions at M. D. Anderson are considered security sensitive; drug screening and thorough background checks will be conducted. The University of Texas M. D. Anderson Cancer Center values diversity in its broadest sense. Diversity works at M. D. Anderson. Smoke-free environment.

Tenure-Track Faculty Position
The Department of Microbiology and Immunology
Medical University of South Carolina
Charleston, South Carolina

The Department of Microbiology and Immunology at the Medical University of South Carolina is seeking applicants for a tenure-track position at the Assistant/Associate level in the areas of microbiology, including biodefense, vaccinology, and microbial or viral pathogenesis of infectious disease or cancer. New faculty will have access to competitive salary and startup funds and benefit from protected time for the establishment of a nationally competitive research program. Appointment requires independent funding.

The Department provides teaching to multiple colleges within the University, and all faculty participate in professional and graduate education as well as maintain an active research program. Laboratories are about to undergo major infrastructural renovations and a number of new faculty will be hired in the near future. The Medical University of South Carolina is a rapidly growing research environment. Extramural research support has consistently increased over the past 10 years, topping $156 million last fiscal year. Major research centers include the Hollings Cancer Center, Gazes Cardiovascular Research Institute, Neuroscience Institute, Children's Research Institute, Center for Aging and the Center for advanced Imaging Research. State-of-the-art research facilities include X-ray crystallography, mass spectrometry, proteomics, microarrays, functional imaging, and confocal microscopy. A BSL-3 small animal/wet lab has been funded and plans are underway for its construction. A major new facility in biomolecular NMR is under development. The Charleston area provides an outstanding quality of life in a historic coastal community offering excellent opportunities in the arts, sports, recreation, and cuisine.

Please send curriculum vitae, state of research interests, and three letters of recommendation addressing both research and teaching potential to: **Microbiology Search Committee, c/o James S. Norris, Ph.D., Department of Microbiology and Immunology, Medical University of South Carolina, 173 Ashley Avenue, PIO Box 250504, Charleston, SC 29425; http://www2.musc.edu/MIC/Micro4803/index.html.**

The Medical University of South Carolina is an Affirmative Action/Equal Opportunity Employer.

THE UNIVERSITY OF SOUTH DAKOTA

VICE PRESIDENT FOR RESEARCH

The Vice President for Research (VPR) will serve as the chief research officer for The University of South Dakota (USD), a doctoral/research intensive university with colleges or schools of arts and sciences, medicine, business, law, education, and fine arts. The university is a multi-campus institution, with the main campus in Vermillion, off-campus work at a variety of locations around the state, and medical school campuses in Vermillion (basic sciences) and Sioux Falls (main clinical site). The university is committed to strengthening its competitive research, technology-transfer, and student research involvement. Within the state of South Dakota and at the university, research has taken on an unprecedented level of priority, due in part to the Governor's 2010 Initiative, which has research as one of five major state goals. South Dakota is an EPSCoR/IdEA state and plays a national leadership role in those programs.

The VPR will oversee all matters related to research and creative scholarship at the university, including sponsored projects administration (preaward), internal research incentive programming, development of research-related policy and budgeting, federal and state relations, undergraduate and graduate student research programming, intellectual property and technology transfer, and research compliance. The VPR reports to the Vice President for Academic Affairs, and is a member of both the President's Executive Committee and the Academic Affairs Working Group. As such the VPR interacts regularly with campus leadership at all levels. The VPR also represents the university on the Research Affairs Council of the Board of Regents, along with the other chief research officers of the system and a newly created system vice president for research. The VPR serves as the principal contact with federal funding agencies and other sources of research funding.

The VPR is a full-time position with a competitive salary based upon experience and potential for taking the university to a new level of research leadership and performance. The successful candidate will be a dynamic leader with intimate knowledge of research from the perspective of a successful, competitively funded researcher as well as a research administrator. An earned doctorate is necessary, preferably a Ph.D., along with a portfolio that demonstrates knowledge of research, leadership skills, creativity, and a high level of productivity.

Send letter of application, resume, names and contact information for three references to: **VPR Search Committee, ATTN: Emery Wasley, Human Resources. University of South Dakota. 414 East Clark Street, Vermillion, SD 57069.**

To receive full consideration, submit resumes before September 30, 2004. Search will continue until the position is filled.

The University of South Dakota is an Equal Opportunity/Affirmative Action Employer committed to increasing the diversity of its faculty, staff and students.

ASSISTANT PROFESSOR OF BIOCHEMISTRY SPECIALIZING IN NMR SPECTROSCOPY

The **DEPARTMENT OF BIOCHEMISTRY** at the **UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT SAN ANTONIO** invites applications for a tenure-track Assistant Professor position in the area of **NUCLEAR MAGNETIC RESONANCE (NMR)** studies of biological macromolecules. Applicants are expected to have expertise in developing and/or applying techniques in NMR spectroscopy to gain insights into macromolecular structure, function, and dynamics. The successful candidate will join a faculty with strong research programs in NMR, X-ray crystallography, and other biophysical approaches to structure/function analysis of proteins (see **http://biochem.uthscsa.edu**) and is part of the expansion of the Department that will occur over the course of the next 3 – 4 years under the new leadership of **Dr. Bruce Nicholson**. Access will be provided to the state-of-the-art NMR equipment recently installed and operated by the Department including Bruker AV 500, 600, and 700 MHz NMR spectrometers and a high sensitivity cryogenically cooled probe for the 600 MHz spectrometer (see **http://nmr.uthscsa.edu**).

San Antonio offers a rich multicultural environment, excellent educational and recreational opportunities, a temperate climate, and a very reasonable cost of living. A generous start-up package will be provided, along with access to a large array of other shared facilities/centers operated by the Department, including those for X-ray crystallography, analytical ultracentrifugation, mass spectrometry, surface plasmon resonance, and others. Candidates will be expected to develop a vigorous, externally funded research program and contribute to teaching in the graduate, medical, or dental curricula. Applicants should send by October 31, 2004 a curriculum vitae, a 1-3 page summary of past and proposed research, and three letters of reference to: **Dr. Andrew P Hinck, Chair of the Search Committee, Department of Biochemistry, MC 7760, Allied Health Building Room 5.206, U. Texas Health Sci. Ctr. at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900**. Questions should be addressed to **hinck@uthscsa.edu**. All faculty appointments are designated as security sensitive positions.

The University of Texas Health Science Center at San Antonio is an Equal Employment Opportunity/Affirmative Action Employer.

MBL
Marine Biological Laboratory

ASSISTANT/ASSOCIATE SCIENTIST
Josephine Bay Paul Center
Program in Molecular Pathogenesis and Global Infectious Diseases

The Program in Molecular Pathogenesis and Global Infectious Diseases in the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution at the Marine Biological Laboratory in Woods Hole invites applications for a position in molecular parasitology. Appointment will be made at the level of Assistant or Associate Scientist (equivalent to the rank of Assistant or Associate Professor). Candidates are expected to develop outstanding, innovative research programs that use modern genomic, computational, genetic, and biochemical approaches to address the basic biology of pathogens and complex host-pathogens interrelationships.

The Josephine Bay Paul Center is a collaborative environment with existing strengths in Molecular Parasitology, Molecular Evolution, Functional Genomics and Microbial Biodiversity. We offer advanced, high throughput facilities for DNA sequencing, DNA microarrays and computational biology. Start-up package and salary is competitive. Initial review of applications will begin immediately and continue until appropriate candidate is identified.

For fullest consideration, please apply by Oct. 31, 2004. Applicants should submit a curriculum vitae, statement of research interests and list of five references to Stephen L. Hajduk, or Mitchell L. Sogin, c/o Tara Nihill, Program in Molecular Pathogenesis and Global Infectious Diseases, Josephine Bay Paul Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543.

An Equal Opportunity/Affirmative Action Employer/Non-smoking workplace

POSITIONS OPEN

St. John Fisher College in Rochester, New York, invites applications for a full-time, tenure-track **ASSISTANT PROFESSOR** position in biology. We seek a candidate with a strong commitment to teaching and undergraduate research. The successful candidate's primary responsibilities will include introductory biology, anatomy/physiology, and upper-level course(s) in an area of interest. Earned Doctorate with experience in molecular biology required; postdoctoral training preferred.

Applicants should submit curriculum vitae, statement of teaching philosophy, research interests, past teaching experience, and a list of references to: **Dr. Mel Wentland, Biology Department, St. John Fisher College, 3690 East Avenue, Rochester, NY 14618. Fax: 585-385-7311; e-mail: mwentland@sjfc.edu.** Applications must be received by November 16, 2004, at which time the review will begin and continue until the position is filled.

To learn more about Fisher, please visit our **website: http://www.sjfc.edu.** *Equal Opportunity Employer/Affirmative Action.*

MOLECULAR AND CELLULAR NEUROSCIENCE RESEARCH

POSTDOCTORAL RESEARCH ASSOCIATE sought to join multidisciplinary molecular and cellular neuroscience group in studies of native or recombinant nicotinic acetylcholine receptors and subunit genetics. Investigations encompass molecular genetic, protein chemical, electrophysiological, immunological, and pharmacological approaches in a unique and dynamic institution. Send curriculum vitae, names and addresses of three references, and brief statement of research experience, interests, and career objectives to: **R. J. Lukas, Ph.D., Division of Neurobiology, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, 350 West Thomas Road, Phoenix, AZ 85013. E-mail: rlukas@chw.edu.** *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

ASSOCIATE PROVOST FOR RESEARCH
Rush University

As a major component of Rush University Medical Center, the largest private academic medical center in Illinois, Rush University integrates patient care, education, and research through the practitioner-teacher model. More than 1,500 research projects—including hundreds of clinical studies to test the effectiveness and safety of new therapies and medical devices—are under way at Rush. Annual research funding has grown from $20 million in 1990 to nearly $70 million today. Some of this important work is housed in the new Robert H. and Terri Cohn Research Building, a state-of-the-art facility where Rush investigators are conducting genetic research to identify the causes of a wide range of diseases.

The Associate Provost for Research will be expected to provide leadership for advancing the research goals of the University and will oversee a major expansion in the base of research funding from federal and state agencies, foundations, and the private sector. The incumbent will have a major role in setting the direction for research in the medical college and other health professional schools; the position entails both academic and management responsibilities.

Candidate qualifications include an M.D., Ph.D., M.D./Ph.D., or other appropriate terminal degree, with a commitment to achieving excellence in research; a strong background in a research-intensive discipline with a clear understanding of the challenges and opportunities facing research faculty; and a distinguished academic background. Also desirable are a record of scholarly achievement, a record of administrative effectiveness, and an acknowledged reputation in research administration.

Please send nominations or applications to: **Eugene Bauer, M.D.** at **e-mail: bauere@kornferry.com,** or mail to: **Korn/Ferry International, 1800 Century Park East, Suite 900, Los Angeles, CA 90067.**

Rush University is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

TENURE-TRACK ASSISTANT PROFESSOR IN POPULATION ECOLOGY

The Department of Zoology, University of British Columbia (UBC), seeks applications for a tenure-track position in population ecology. We encourage individuals who conduct field research on animal populations, preferably terrestrial vertebrates or intertidal animals, to apply. Duties include developing a strong research program, teaching courses in ecology or organismal biology and actively participating in the UBC Biodiversity Research Centre.

Salary will be commensurate with experience. Appointment will be at the Assistant Professor level and is subject to final budgetary approval.

Applicants should send curriculum vitae, summary of research interests and teaching philosophy, and reprints of three key publications. Letters should be sent directly from three referees. Address all materials to: **Dr. Bill Milsom, Head, Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC, Canada, V6T 1Z4 (e-mail: head@zoology.ubc.ca, fax: 604-822-5780).** Applications accepted up to 20 September 2004, or until a suitable candidate is found.

The University of British Columbia hires on the basis of merit and is committed to employment equity. All qualified applicants are encouraged to apply; however, in accordance with Canadian immigration requirements, Canadian citizens and permanent residents of Canada will be given priority.

POSTDOCTORAL POSITION available immediately to understand the mechanism and dynamics of adenosine triphosphate-driven DNA packaging in viruses using bacteriophage T4 model. Novel combinatorial mutagenesis, biochemical, and structural approaches will be used. Strong background in protein biochemistry desired. Send curriculum vitae and names of three references to: **Dr. Venigalla Rao, Department of Biology, Catholic University, 620 Michigan Avenue, N.E., Washington, DC 20064. E-mail: rao@cua.edu.**

STANFORD UNIVERISTY
SCHOOL OF MEDICINE
department of
GENETICS

invites applications for a tenure-track position at the ASSISTANT/ASSOCIATE PROFESSOR level.

We seek highly interactive candidates with an interest in teaching and an outstanding record of research achievement in any area of genetics including, but not limited to, human genetics or genomics and pathophysiology of genetic disease.

Candidates should have a Ph.D. and/or M.D. degree and postdoctoral research experience. The new faculty member is expected to participate in the teaching of graduate and medical students, and to establish an independent research program that strengthens the diverse research interests of the current departmental faculty members (*http://genetics.stanford.edu)*.

Stanford University is an equal opportunity, affirmative action employer. Candidates are encouraged to apply electronically by October 31, 2004 with curriculum vitae, a statement of their research interests, teaching experience and future research plans, and the names, phone numbers, email and postal addresses of three or more individuals willing to serve as references, to:

Uta Francke M.D.
Professor of Genetics and Pediatrics
Stanford University School of Medicine
Beckman Center for Molecular and Genetic Medicine B203
Stanford, CA 94305-5323
email: faculty-search-z474@genome.stanford.edu

Department of Health and Human Services
National Institutes of Health
The National Cancer Institute
Center for Cancer Research

In collaboration with The National Heart, Lung, and Blood Institute, The National Institute of Allergy and Infectious Diseases, and The National Institute of Diabetes and Digestive and Kidney Diseases has formed graduate education partnerships with North Carolina State University, Michigan State University, The University of Illinois, and The University of Maryland to offer **combined comparative pathology and biomedical research training for doctors of veterinary medicine** designed to address translation of research findings from animal models to the clinical setting. Following initial pathology and graduate training at a university partner, trainees transfer for additional training within the intramural research laboratories of the NCI, NHLBI, NIAID, or NIDDK. The training initiative provides for multidisciplinary training in animal pathophysiology, rodent or NHP pathology, human pathology, molecular biology, and medical research. The program leads to a Ph.D. and eligibility for certification as a veterinary pathologist. Support includes attractive stipend, tuition and health insurance benefits, and other training assistance. The CCR website **http://ccr.nci.nih.gov/resources/training/default.asp** offers detailed descriptions of the programs and other links to important information. Candidate veterinarians must be U.S. citizens or U.S. permanent residents, and have less than five years postdoctoral experience.

Apply on-line for fellowships beginning July 1, 2005, with up to 5 years support, through the NIH Graduate Partnership Program at **http://gpp.nih.gov/**. On-line applications are due by **December 2, 2004**. For consideration, separate application must also be made directly to partnership university graduate schools of choice. For additional information contact either **Dr. Jonathan S. Wiest** at **wiestj@mail.nih.gov** or **Dr. R. Mark Simpson**, at **ncimolpathol@mail.nih.gov**.

DHHS and NIH are Equal Opportunity Employers.

FACULTY POSITIONS
Biological Sciences

The Department of Biological Sciences at the University of Pittsburgh invites applications for two full-time tenure-track faculty appointments, pending budgetary approval. Appointments are anticipated to begin in September 2005. Our department has a broad-based, interactive group of researchers whose interests encompass nearly every area of modern biology. We are seeking colleagues from any discipline; those with interests in gene expression, chromatin structure/function, genomics, host-pathogen interactions, cell or developmental biology, bioinformatics, biochemistry or biophysics are especially encouraged to apply. These appointments are expected to be made at the **ASSISTANT PROFESSOR** level, but experienced candidates with outstanding records will be considered for appointment at higher ranks. The successful candidates must have a Ph.D. and extensive postdoctoral experience and will be expected to establish extramurally funded research programs, train graduate students, and participate in undergraduate education. To ensure full consideration, applications should be received by **November 1, 2004**. Applicants should send curriculum vitae along with a summary of research interests and goals as a single electronic document (PDF format preferred) to **biojobs@pitt.edu**. In addition, applicants should arrange to have at least three letters of reference sent to:

Search Committee
Department of Biological Sciences
University of Pittsburgh
Pittsburgh, PA 15260
(412) 624-4266

Further information on the Department of Biological Sciences is available at: **http://www.pitt.edu/~biology**.

The University of Pittsburgh is an Affirmative Action/Equal Opportunity Employer. Women and members of minority groups underrepresented in academia are especially encouraged to apply.

UNIVERSITY of DAYTON

Biomedical Tenure-Track Position-Assistant Professor

The Department of Biology at the University of Dayton, OH, USA, seeks applicants for a tenure-track Assistant Professor position, beginning August 2005. Requirements include a Ph.D., relevant post-doctoral experience, and a commitment to excellence in research and teaching. We seek an individual with primary research interest in mammalian physiology, cell biology, tissue regeneration, bionanotechnology or stem cell biology. The successful candidate is expected to develop an extramural funded research program that will complement existing faculty in the above areas, and to involve Ph.D., M.S. and undergraduate students in his/her research program. Teaching expectations will be one lecture course per semester in mammalian physiology or cell biology, and supervision of the laboratory component. Start-up funds are available to establish a new research laboratory in the candidate's area of expertise.

To apply, send curriculum vitae, statements of research direction and teaching interests, selected reprints, and at least three letters of recommendation to: **Dr. Marie-Claude Hofmann, Chair of the Search Committee, Department of Biology, University of Dayton, 300 College Park, Dayton, OH 45469-2320, USA**. The committee will begin reviewing the applications by **October 25, 2004**, and the search will continue until the position is filled.

The University of Dayton is a private comprehensive university located in the Columbus-Dayton-Cincinnati (Ohio) metroplex area. Please visit our web sites: **http://biology.udayton.edu** or **http://artssciences.udayton.edu/Biology/** for further information.

The University of Dayton, a comprehensive Catholic University founded by the Society of Mary in 1850, is an Equal Opportunity/ Affirmative Action Employer. Women, minorities, individuals with disabilities and veterans are strongly encouraged to apply. The University of Dayton is firmly committed to the principle of diversity.

PEGGY M. PENNINGTON COLE ENDOWED CHAIR in Maternal Biology and the Risk of Obesity

The Pennington Biomedical Research Center is an internationally recognized nutritional research center. Its 70-member faculty apply multidisciplinary approaches to understanding the interactions between nutrition, health, and chronic disease primarily as it relates to obesity, diabetes, cardiovascular disease, cancer, and aging. As a research center, its teaching and training is limited to mentoring of graduate students and postdoctoral fellows. The Center has made significant investments in a range of cutting-edge technologies including metabolic chambers, metabolic kitchen facilities, mass spectrometry laboratories, confocal microscopy suite, genomics, proteomics, microarrays, and transgenics and has both inpatient and outpatient facilities for clinical studies. The main research facility has about 400,000 square feet of research space. Further information on current faculty and their research may be found at **website: http://www.pbrc.edu.** All faculty appointments are academic appointments within the Louisiana State University System.

The Pennington Biomedical Research Center invites qualified applicants to apply for a **FULL PROFESSOR POSITION** to be appointed to the Peggy M. Pennington Cole Endowed Chair in Maternal Biology and the Risk of Obesity. The chair holder is anticipated to have an M.D., Ph.D., or equivalent qualifications but would be expected to use basic science approaches to understand mechanisms underlying the fetal and maternal effects on health of the offspring and on subsequent risks of adulthood diseases. The chair holder will have an international reputation as an accomplished researcher, a well-funded research program, a strong publication record, and leadership abilities. The chair holder will have demonstrated ability to work well with other scientists in collaborative research. The overarching aim will be to study the mechanisms through which nutritional and environmental influences during pregnancy, lactation, and the neonatal period influence the risk for development of obesity and its co-morbidities during adult life. Identification of markers that can subsequently be used in human populations to identify risk status of human infants is a likely outcome of this research.

Applications, including cover letter, curriculum vitae, bibliography, names, addresses, and telephone numbers of three references will be accepted until the position is filled. Send to: **Claude Bouchard, Ph.D., Executive Director, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808. Telephone: 225-763-2513; fax: 225-763-0935.** *Louisiana State University System/Pennington Biomedical Research Center is an Equal Opportunity/Affirmative Action Employer.*

FACULTY POSITION Biological Mechanism MIT, Department of Biology

MIT is seeking an outstanding scientist with a strong record of research accomplishment for a tenure-track position. Applicants at all faculty levels will be considered. A successful applicant will be expected to have or develop a significant and independent research program and have a commitment to excellence in undergraduate and graduate education.

Applicants should submit curriculum vitae, including a summary of current and proposed research programs, and should arrange for three letters of recommendation to be sent to:

Biological Mechanism Search Committee
Attn: Tania Baker
MIT 77 Massachusetts Avenue
Room 68-523
Cambridge, MA 02139

Consideration of completed applications will begin on November 15, 2004.

MIT is an Affirmative Action/Equal Opportunity Employer. Qualified women and minority candidates are especially encouraged to apply.

POSITIONS OPEN

TENURE-TRACK FACULTY POSITIONS Department of Cellular and Integrative Physiology Indiana University School of Medicine

The Department seeks applicants for three tenure-track positions. Academic rank will be commensurate with experience. Applicants must have an M.D. or Ph.D. degree, at least three years of postdoctoral experience, high-quality peer-reviewed publications, evidence of independent research, and competitive funding potential. We seek innovative scientists using integrated molecular, cellular, and whole animal approaches to study important physiological questions. All areas of research will be considered, but preference will be given to candidates who complement strengths in the Department in smooth muscle, cytoskeleton, mechanotransduction, growth, apoptosis, renal physiology, membranes (ion channels, caveolae, adhesion junctions), diabetes, imaging, and computational biology. Successful applicants will be expected to maintain an extramurally funded research program and participate in the teaching of medical and graduate students. Significant resources available include competitive startup packages, doubling of newly renovated laboratory space, and long-term research and salary incentives. This is the first phase of growth in the Department which is expected to include six-to-eight new positions in the next four-to-five years. Further information can be found at **website: http://www.iupui.edu/~medphys.** Review of applications will begin immediately and the deadline for receipt is October 12, 2004.

Applicants should send (preferably in electronic format) their curriculum vitae, brief statement of research interests and goals, and the names of three references to**: Dr. Michael Sturek, Chair, Department of Cellular and Integrative Physiology, c/o Marlene Brown (e-mail: mbrown3@iupui.edu), 635 Barnhill Drive, M.S. 309, Indianapolis, IN 46202-5120.** *Indiana University is an Equal Employment Opportunity/Affirmative Action Employer.*

STAFF AND POSTDOCTORAL POSITIONS LNLS/ABTLuS–BRAZIL

The Brazilian Synchrotron Light Laboratory (LNLS) is expanding its scientific and technical staff. The following qualified people are invited to apply for both visiting and staff positions:

Researchers (code RSCR-SYN) with postdoctoral experience in synchrotron X-ray scattering and/or absorption spectroscopy, vacuum ultraviolet spectroscopy, or surface science. Scientists and engineers interested in the development of insertion devices, synchrotron light instrumentation, microfabrication, and light sources. Postdoctoral candidates (code PDOC-SYN) with previous experience in synchrotron light research as well as outstanding candidates without this experience but willing to work in the areas above.

Researchers (code RSCR-BIO) with postdoctoral experience in the area of biophysics including protein and peptide nuclear magnetic resonance, structure-driven drug design, protein chemistry, folding, modeling, crystallography, and molecular biology, and Postdoctoral candidates (code PDOC-BIO) in the areas above.

The LNLS offers an intellectually stimulating scientific environment and state-of-the-art facilities. Successful candidates will be expected to show an outstanding research record in science and/or technology.

Application deadline is November 30, 2004. Please refer to the code when applying.

Send resume, list of publications, and the names of three references to: **Director General, LNLS/ABTLuS, C.P. 6192, 13084-971 Campinas (SP), Brazil** or **e-mail: research@lnls.br. Website: http://www.lnls.br.**

POSITIONS OPEN

Department of Chemistry. DePauw University. Biochemistry or bio-organic chemistry. Applications invited for **TENURE-TRACK POSITION** beginning August 2005. Ph.D. preferred, all but dissertation required. Commitment to teaching and research with undergraduates required. Prior teaching or postdoctoral experience desired. Successful candidate will teach biochemistry and organic chemistry in Department's introductory core and advanced courses in specialty area, and will be encouraged to develop course for non-majors. Commitment to develop vigorous research program involving undergraduate biochemistry majors required. DePauw has exceptional programs for supporting its faculty members, including a pre-tenure leave and funding for professional and curriculum development activities (see **website: http://www.depauw.edu/admin/acadaffairs/facdev.htm**). Matching funds and other incentives will be provided to support search for external funding. Submit letter of application, curriculum vitae, three letters of recommendation, graduate and undergraduate transcripts, statements of teaching and research interests and teaching philosophy, and evidence of teaching effectiveness to: **Professor Bryan Hanson, Search Chair, Department of Chemistry, DePauw University, Greencastle, IN 46135.** Materials may be submitted electronically to **e-mail: hanson@depauw.edu.** Review of applications begins October 1, 2004, and continues until position is filled. *DePauw University is an Affirmative Action/Equal Opportunity Employer. Women and members of underrepresented groups are encouraged to apply.*

ASSISTANT PROFESSOR Systems Visual Neuroscience

The University of California at Berkeley invites applications for a tenure-track position in the School of Optometry at the Assistant Professor level beginning in the fall of 2005, with a joint appointment in the Helen Wills Neuroscience Institute (50 percent Optometry and 50 percent HWNI). Applicants should hold a doctoral degree in cognitive neuroscience, neuroscience, psychology, vision science, or a related field. We are particularly interested in candidates employing single unit neurophysiology or imaging (either optical imaging or functional magnetic resonance imaging) to study the neural mechanisms of vision. Applications must be postmarked by November 15, 2004, and should include a resume, selected publications, a brief statement of research interests and future plans. Send applications to: **Chair, Faculty Search Committee, Systems Visual Neuroscience, School of Optometry, University of California, Berkeley, CA 94720-2020.** Candidates should also arrange to have at least three letters of recommendation sent to the above address, and request that references read the University's statement on confidentiality (**website: http://apo.chance.berkeley.edu/evalltr.html**) prior to submitting their letters. Applications postmarked after November 15, 2004, cannot be considered. *The University of California is an Equal Opportunity/Affirmative Action Employer. All qualified applicants including minorities and women are encouraged to apply.*

The Center of Marine Biotechnology of the University of Maryland Biotechnology Institute invites applications for a tenure-track **ASSISTANT/ASSOCIATE PROFESSOR** position in fish immunology. Ph.D. with postdoctoral training in the application of molecular/biochemical approaches to understanding humoral or cellular immunity preferred. Candidates must demonstrate strong research record, including publication of seminal papers on structure/function of fish immune system. Applicants should submit letter of intent describing specific interest, experience, overall qualifications, future plans and career goals, with detailed curriculum vitae, including list of publications, and names and addresses of at least three professional references. Packages should be sent no later than October 30, 2004, to: **Search Committee Chair, Fish Immunology, Center of Marine Biotechnology, 701 East Pratt Street, Suite 236, Baltimore, MD 21202-3101.**

FACULTY POSITION IN BIOPHYSICS
The Johns Hopkins University

The Thomas C. Jenkins Department of Biophysics seeks candidates for the first of **TWO TENURETRACK/TENURED FACULTY POSITIONS** in structural Biology/macromolecular function. We are particularly interested in candidates with expertise in NMR and X-ray crystallography who apply these methods to the mechanism of biological regulation. Candidates should have a strong background in the physical sciences. Faculty using NMR will participate in an expanding interdepartmental NMR center which includes faculty from Chemistry and Biology. The Biophysics Department anticipates significant growth during the next several years, including a new building and additional faculty appointments. Positions are at any rank. The first position is available immediately; the successful candidate will participate in recruitment for the second position, which becomes available the following year.

Please send by **15 December 2004** a cover letter, CV, and brief description of your research plans to: **Faculty Search Committee, T. C. Jenkins Department of Biophysics, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218-2685; tel. 410-516-7245**. Candidates should arrange for three letters of reference to be sent to the same address.

The Johns Hopkins University is an Affirmative Action/Equal Opportunity Employer.

National Center Director
National Center for Environmental Research
Office of Research and Development

EPA's Office of Research and Development is seeking a highly qualified science manager to direct the National Center for Environmental Research (NCER).

Applicants must have a Bachelors degree, or higher, in a discipline related to environmental science or engineering with demonstrated ability to understand a multidisciplinary program with national environmental policy implications. In addition to possessing core executive qualifications of the Senior Executive Service, the individual must demonstrate highly creative problem solving skills and exceptional ability to communicate science issues, results, and impacts to a broad range of audiences and stakeholders including EPA program managers, other federal agencies, Congress, and other science organizations.

For an overview of NCER's activities, please go to www.epa.gov/ncer.

This is a Senior Executive Service position. The position opens on July 19, 2004 and closes September 30, 2004. The salary range is $104,927 to $145,600 depending on qualifications.

Applicants must address the executive and technical qualifications described in the vacancy announcement for this position. Call or write to USEPA/OARM/OHR/SES Human Resources Staff (3650A), 1200 Pennsylvania Avenue, N.W., Washington, D.C. 20460. You may download a full announcement at www.usajobs.opm.gov. The vacancy number is EPA-04-SES-ORD-6322.

U.S. Citizenship Required.
Applications must be received by the closing date of September 30, 2004.
EPA is an Equal Opportunity Employer.

FACULTY POSITIONS in BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF IOWA
ROY J. AND LUCILLE A. CARVER COLLEGE OF MEDICINE

The Dept. of Biochemistry of the Univ. of Iowa seeks highly qualified applicants for one or more tenure track faculty positions at any rank. The department has broad interests (www.biochem.uiowa.edu) and current faculty have strong collaborative interactions with colleagues throughout the Carver College of Medicine and the University. They participate in multiple interdisciplinary training programs including the Center for Biocatalysis and Bioprocessing, Genetics, Molecular Biology, Molecular Parasitology and the Medical Scientist Training Program. Outstanding new and renovated research space with excellent shared instrumentation is available. We welcome applicants with research interests in any area of biochemistry including cellular, developmental and molecular biology. All applicants must have a relevant graduate degree (Ph.D., M.D., or the equivalent) and productive postdoctoral experience. They will be judged on their potential to initiate and maintain a vigorous, independent research program, to teach and to train students and postdoctoral fellows.

Applications should include a complete CV, a 3-page summary of research accomplishments and future plans, and a brief statement of teaching interests. These should be sent as a single pdf file to biochemposition@uiowa.edu, with the applicant's last name in the filename; reprints in pdf format may be sent with the application. Applicants for the rank of Assistant Professor should ask three scientists familiar with their qualifications to submit letters of reference, preferably to biochemposition@uiowa.edu. Applicants at this rank also will be considered for nomination to the Univ. of Iowa Presidential Biological Scholar Program (www.uiowa.edu/~pbschol). Senior candidates should provide names of 3 references. Consideration of completed applications will begin on October 15, 2004. Questions may be directed to Prof. Madeline A. Shea, Chair of the Faculty Search Committee, Dept. of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, Univ. of Iowa, Iowa City, IA, 52242-1109, phone 319-335-7933, fax 319-335-9570. *The University of Iowa is an Affirmative Action/Equal Opportunity Employer. Women and minorities are strongly encouraged to apply.*

Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina

COMPUTATIONAL CHEMIST
Laboratory of Structural Biology

The Laboratory of Structural Biology (LSB) of the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, is seeking a computational chemist to serve as a staff scientist in LSB. The successful candidate will work very closely with a senior member of the LSB and will provide computational chemistry expertise to the LSB. Applicants should have a Doctoral degree in physical or theoretical chemistry (or equivalent) with expertise in computational chemistry and demonstrated skills in a) computer modeling (chemistry, biochemistry), including 3D graphics, molecular mechanics and dynamics, docking, quantum mechanics/chemistry), b) computer programming (Fortran and/or C essential, other languages desirable), c) managing computer hardware (single processor, multiprocessor, networks, printers), working knowledge of UNIX, LINUX, WINDOWS and other OS, and ability to evaluate, port and install modeling and visualization software d) helping to write scientific papers for quality journals, e)working in group efforts as well as working largely independently towards common goals. The level of appointment will be commensurate with the selectee's background. For additional information about this position, contact Dr. Thomas Darden, Head, Molecular Modeling Group, at 919-541-4933 (darden@niehs.nih.gov).

Interested persons should send their curriculum vitae with a statement of research interests, and arrange for three letters of recommendation to be sent by **November 15, 2004**, to:

Ms. Cindy Garrard (Vacancy DIR04-08)
National Institutes of Health
National Institute of Environmental Health Sciences
P.O. Box 12233, Maildrop A2-06
Research Triangle Park, NC 27709
dir-appls@niehs.nih.gov

DHHS and NIH are
Equal Opportunity Employers

ASSISTANT OR ASSOCIATE PROFESSOR (RESEARCH) IN SLEEP RESEARCH
Sleep Research Center
Department of Psychiatry and Behavioral Sciences
Stanford University School of Medicine

The Department of Psychiatry and Behavioral Sciences at the Stanford University School of Medicine is recruiting for the full-time Research Faculty position in sleep research. This is a nontenure contract position at the level of Assistant/Associate Professor (Research) in the School of Medicine, and is co-terminus with funding. Applicants for this position must have a Ph.D., M.D., or equivalent degree. They must be recognized by the scientific community as possessing a superior level of expertise in the neurobiology of sleep and basic biological rhythms. A proven record of investigative productivity in basic science related to the neurobiology of sleep, electroencephalography (EEG), and its circadian timing is required. Experience in fundraising and directing a multidisciplinary research group/program is desirable. The selected candidate will direct an ongoing multidisciplinary research program to investigate the underpinnings of circadian and homeostatic elements of sleep-wake regulation and the EEG. Expertise and interest in basic or clinical sleep research, whether in humans and/or animal models is required. The applicant will be expected to collaborate with other members of the Sleep Research Center in the utilization of the existing large-scale rodent sleep research facility and participate in joint projects. Candidates should demonstrate an interest in and commitment to teaching including graduate/medical students, residents, and postdoctoral fellows.

Applicants should forward curriculum vitae and the names of three references within four weeks of the date of publication of this advertisement to:

Dr. Emmanuel Mignot
Chairman of the Search Committee
Department of Psychiatry and Behavioral Sciences
Stanford University School of Medicine
Sleep Research Center
701 Welch Road, Suite 2226
Stanford, CA 94304

Stanford University is an Equal Opportunity/Affirmative Action Employer.

TENURE-TRACK BIOLOGY FACULTY

Department of Biology, University of Wisconsin-La Crosse (UW-La Crosse), invites applications for two, academic year, tenure-track positions at the level of **ASSISTANT PROFESSOR** for fall 2005.

Plant Ecologist: Teach plant biology, a course in area of expertise, and participate in teaching one or more of the following: ecology, introductory biology, plant-microbe interactions.

Comparative Animal Physiologist: Teach comparative animal physiology or environmental physiology, a course in area of expertise, and participate in teaching one or more of the following: human anatomy and physiology, vertebrate zoology, and introductory biology.

Ph.D. in a biological science is required. Must have a strong commitment to undergraduate education. Some previous teaching experience is desirable. Will be expected to develop an externally funded research program and direct undergraduate and graduate (M.S.) research. Positions contingent upon availability of funding. Submit letter of application, curriculum vitae, statements of teaching philosophy and research interests, graduate and undergraduate transcripts, and three letters of recommendation by November 1, 2004, to: **Dr. Mark Sandheinrich, Department of Biology, University of Wisconsin-La Crosse, La Crosse, WI 54601. Website: http://www.uwlax.edu/vacancies/.**

UW-La Crosse is an Affirmative Action/Equal Opportunity Employer. Women, persons of color, and individuals with a disability are encouraged to apply. "Reasonable accommodations" are provided for applicants with disabilities.

POSITIONS OPEN

ASSISTANT PROFESSOR, HOST-PATHOGEN INTERACTIONS
Department of Plant Pathology
Michigan State University
East Lansing, Michigan

We seek to hire a tenure-system, academic-year appointment faculty member at the Assistant Professor level who will use integrative and molecular approaches to study important problems in host-pathogen interactions. The candidate is expected to establish an internationally recognized research program on host-pathogen interactions involving any major group of plant pathogens. Research emphasis may range from functional genomics, proteomics, bioinformatics, sub-cellular imaging, molecular basis of pathogenicity and/or disease susceptibility/resistance of plant-pathogen interactions, or pathogen genetics/biology; the approach should contribute to an understanding of molecular events in the interactions of plants and pathogens resulting in plant resistance or plant disease. The successful candidate will also participate in graduate and/or undergraduate teaching.

The Department of Plant Pathology has 14 tenure-system faculty. Research facilities include modern laboratory and plant growth facilities. Michigan State University has a large and vibrant community of plant biologists with research interests spanning all major areas in the plant sciences. State-of-the-art analytical core facilities include the Genomics Technology Support Facility, Mass Spectrometry/Proteomics Facility and Center for Advanced Microscopy. Because of the opportunity for synergistic appointments, we encourage applicants from candidates who are present or potential collaborators. There is an option for a joint appointment in biochemistry and molecular biology, crop and soil sciences, forestry, horticulture, or plant biology. Candidates must possess a Ph.D. and have graduate and/or postdoctoral research experience in plant pathology with demonstrated productivity and evidence of potential for independent research. The application should include: cover letter, curriculum vitae, statement of research interests and future directions, and three or more letters of reference. *Nonresidents of the United States must satisfy requirements necessary for issuance of a U.S. work permit prior to the date of appointment.* Application materials should be addressed to:

Dr. George W. Sundin
Department of Plant Pathology
Michigan State University
East Lansing, MI 48824-1311
Telephone: 517-355-4573
E-mail: sundin@msu.edu

Closing date for applications is October 31, 2004, but search will continue until a suitable candidate is identified. *Michigan State University is an Affirmative Action/Equal Opportunity Employer.*

A position is available for a nontenure-track **ASSISTANT/ASSOCIATE RESEARCH PROFESSOR** within the Wells Center for Pediatric Research at the Indiana University School of Medicine. Candidates must have Ph.D. or M.D. degrees, and a strong background in molecular and cellular biology, including experience with murine embryo development. The ideal candidate will have experience in hematopoietic stem and progenitor cell biology and confocal laser microscopy. Expertise in the method of whole embryo in vitro culture is also desirable. Applicants should send current curriculum vitae and contact information for at least three professional references to:

Mervin C. Yoder, M.D.
Professor of Pediatrics
and Biochemistry and Molecular Biology
Herman B. Wells Center for Pediatric Research
1044 W. Walnut
Building R4-Room 402E
Indianapolis, IN 46202
E-mail: myoder@iupui.edu

Indiana University is an Equal Employment Opportunity/Affirmative Action Employer, Minorities/Females/Persons with Disabilities.

POSITIONS OPEN

FACULTY POSITIONS IN NEW CARDIOVASCULAR RESEARCH INSTITUTE

As part of its $500 million 10-year initiative, the University of Rochester School of Medicine has purchased an 88,000 square foot building which will become home to a new Cardiovascular Research Institute. The Institute will build upon a well-established research base in the areas of vascular biology, ischemia-reperfusion injury, molecular pharmacology, signal transduction, and cardiovascular genetics (see **website: http://www.urmc.rochester.edu/Aab/Cardio.htm**). Competitive startup packages are presently available for two developmental biologists at the **JUNIOR FACULTY** level. The Institute is particularly interested in outstanding candidates with demonstrable promise in the area of zebrafish and mouse cardiovascular development. Qualified applicants should send curriculum vitae, research plan, and three letters of recommendation to:

Faculty Search Committee,
Cardiovascular Research Institute
Box 679
University of Rochester Medical Center
601 Elmwood Avenue
Rochester, NY 14642
Fax: 716-273-1497

University of Rochester is an Equal Opportunity Employer, committed to workforce diversity.

The Biology Department of Gonzaga University invites applications for a tenure-track **ASSISTANT PROFESSOR** position. We seek to hire a broadly trained biologist who will complement the existing faculty. The position will begin fall 2005. Teaching assignments may include Diversity of Life (BIOL101), as well as upper-division courses in area of specialization and courses for nonscience majors or K-12 educators. The area of specialization is open, but areas of particular interest to the Department include microbial ecology/evolution, entomology, or animal physiology. To receive full consideration, applications and three letters of recommendation should be received by 1 November 2004. Applications should consist of cover letter, curriculum vitae, statement of research interests and plans for undergraduate participation, and statement of teaching philosophy and interests. Applications and letters should be sent to: **Dr. Nancy Staub, AD Box 6, Gonzaga University, 502 E. Boone Avenue, Spokane, WA 99258.** For further information about applying for this position and a temporary spring 2005 position, see our **website: http://gonzology.gonzaga.edu/faculty-staff/jobs/.** *Gonzaga University is a Jesuit, Catholic, humanistic university looking for candidates who can contribute to its educational needs and missions. Gonzaga is an Affirmative Action/Equal Opportunity Employer seeking to increase its diversity.*

MOLECULAR BIOLOGIST

The Department of Biology, College of Charleston, invites applications for a tenure-track position in molecular biology at the **ASSISTANT PROFESSOR** level. Candidates must possess a Ph.D., a strong commitment to teaching, and an active research program with the potential for undergraduate involvement. Teaching responsibilities include an upper-level lecture and laboratory course in molecular biology as well as an upper-level course in the candidate's area of specialization. Advising premedical students is also expected. The College of Charleston is a public liberal arts and sciences institution of 10,000 students. The College's primary aims are teaching and research excellence. In addition to its undergraduate programs, the Department offers M.S. degrees in marine biology and environmental studies. Information about the Biology Department can be found at **website: http://www.cofc.edu/~biology/.** Applicants should submit curriculum vitae, statements of teaching philosophy and research interests, reprints of recent publications, and three letters of reference by 1 November 2004 to: **Chair, Department of Biology, College of Charleston, Charleston, SC 29424.** *The College of Charleston is an Equal Opportunity/Affirmative Action Employer and encourages the applications of qualified women and minorities.*

TEMPLE UNIVERSITY
DEPARTMENT OF CHEMISTRY
ORGANIC CHEMISTRY FACULTY POSITION

The Department of Chemistry at Temple University invites applications and nominations for a tenured/tenure-track faculty position in the area of **Organic Chemistry**. The position is open with respect to rank. Applicants at the Assistant Professor level are expected to demonstrate strong potential for establishing a vigorous research program funded by peer-reviewed research grants and for developing excellence in teaching. Applicants at the Associate and Full Professor levels are expected to have established research programs of high quality, supported by substantial externally funded peer-reviewed research grants, and demonstrated significant teaching accomplishments. Salaries are highly competitive and substantial resources have been provided for startup funding. Ample modern laboratory space is available.

The Department of Chemistry (**http://www.chem.temple.edu**) is engaged in a new initiative of growth in research and education, and enjoys multiple collaborative interactions with research groups in other departments in the College of Science and Technology, the College of Engineering, and the Health Sciences campus. Temple University (**http://www.temple.edu**), located in historic Philadelphia, is part of the Pennsylvania Commonwealth System of Higher Education, and serves over 34,000 students. Philadelphia is a vibrant center of the arts and sciences and a major locus of chemical, biomedical, pharmaceutical and biotechnological research and development.

Applicants should submit curriculum vitae; a statement of research interests and (if applicable) current grant support; a statement of teaching philosophy; and arrange to have four letters of recommendation sent to: **Dr. Robert J. Levis, Professor and Chair, Department of Chemistry (016-00), Temple University, Beury Hall, 13th and Norris Streets, Philadelphia, PA 19122**. Review of applications will begin immediately and will continue until suitable candidates are identified.

Temple University is an Equal Opportunity/Affirmative Action Employer. The Department specifically invites and encourages applications from women and minorities.

TEMPLE UNIVERSITY
DEPARTMENT OF CHEMISTRY
BIOCHEMISTRY/CHEMICAL BIOLOGY
FACULTY POSITION

The Department of Chemistry at Temple University invites applications and nominations for a tenured/tenure-track faculty position in the area of **Biochemistry/Chemical Biology**. The position is open with respect to rank. Applicants at the Assistant Professor level are expected to demonstrate strong potential for establishing a vigorous research program funded by peer-reviewed research grants and for developing excellence in teaching. Applicants at the Associate and Full Professor levels are expected to have established research programs of high quality, supported by substantial externally funded peer-reviewed research grants and demonstrated significant teaching accomplishments. Salaries are highly competitive and substantial resources have been provided for startup funding. Ample modern laboratory space is available.

The Department of Chemistry (**http://www.chem.temple.edu**) is engaged in a new initiative of growth in research and education, and enjoys multiple collaborative interactions with research groups in other departments in the College of Science and Technology, the College of Engineering, and the Health Sciences campus. Temple University (**http://www.temple.edu**), located in historic Philadelphia, is part of the Pennsylvania Commonwealth System of Higher Education, and serves over 34,000 students. Philadelphia is a vibrant center of the arts and sciences and a major locus of chemical, biomedical, pharmaceutical and biotechnological research and development.

Applicants should submit curriculum vitae; a statement of research interests and (if applicable) current grant support; a statement of teaching philosophy; and arrange to have four letters of recommendation sent to: **Dr. Robert J. Levis, Professor and Chair, Department of Chemistry (016-00), Temple University, Beury Hall, 13th and Norris Streets, Philadelphia, PA 19122**. Review of applications will begin immediately and will continue until suitable candidates are identified.

Temple University is an Equal Opportunity/Affirmative Action Employer. The Department specifically invites and encourages applications from women and minorities.

Faculty Positions in Neuroscience, Cell and Developmental Biology
Department of Cell Biology
Emory University School of Medicine

Applications are invited for tenure-track faculty positions at the ASSISTANT, ASSOCIATE and FULL PROFESSOR levels. The Department is interested in strengthening its programs in cell, developmental and neurobiology. All outstanding candidates are encouraged to apply, although particular areas of interest include:

- **molecular and cellular neuroscience, and**
- **the use of model genetic systems to study basic biomedical problems.**

Emory University is in the midst of a major growth initiative to be one of the premier research institutions in the nation. Within the past seven years, eight new investigators, including a new Chair, have joined the Department of Cell Biology, which recently relocated into the new Whitehead Biomedical Research Building. Virtually all other departments in the School of Medicine are experiencing similar growth, with the recruitment of new Chairs, faculty and occupancy of new physical facilities. Emory bioscience faculty participate in one or more of eight rapidly growing, interdisciplinary predoctoral training programs. For questions contact: **search@cellbio.emory.edu**. Interviews begin January 2005.

Send *curriculum vitae*, research plan, representative reprints and three reference letters by **December 1, 2004**, to:

Chair, Search Committee
Department of Cell Biology
Emory University School of Medicine
Whitehead Research Building, Room 400
615 Michael Street
Atlanta, GA 30322

Emory University is an Equal Opportunity Employer. Women and members of underrepresented racial and ethnic groups are encouraged to apply.

Department of Health and Human Services
National Institutes of Health
National Cancer Institute

Program Manager, Cancer Nanotechnology

The Office of the Director of the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS) invites applications for a Program Manager, Cancer Nanotechnology in the Office of Technology and Industrial Relations. This is a key position responsible for providing leadership, management, and oversight in the field of nanotechnology to lead programmatic development at NCI. NCI is seeking a visionary leader of national stature to coordinate the implementation of the NCI cancer nanotechnology plan (CNPlan).

The individual selected for this position will possess an M.D. or Ph.D. degree (or have equivalent training and experience) with independent research and administrative experience and a publication record in the application of nanotechnologies for biomedical purposes. In addition, individual will have experiences in materials development/synthesis/fabrication, physical/chemical properties of nanomaterials and nanodevices, prototyping and beta-testing.

Salary is commensurate with research experience and accomplishments, and a full Civil Service package of benefits (including retirement, health, life and long-term care insurance, Thrift Savings Plan participation, etc.) is available.

The NCI vacancy announcement for this position contains complete application procedures and lists all mandatory information which you must submit with your application. To obtain the vacancy announcement for this position which will be available on or about September 17, 2004 and posted under announcement number **NCI-04-3109**, you may visit the NIH Career website at: **http://careerhere.nih.gov** OR you can have it faxed to you by calling **1-800-728-JOBS** (**for local calls, 301-594-2953**). Applications must be postmarked by **October 29, 2004**.

NCI, NIH and DHHS are Equal Opportunity Employers.

TENURED FACULTY POSITION
University of California, Irvine
Department of Chemistry

The Department of Chemistry and the Program in Pharmaceutical Sciences at the University of California, Irvine (UCI), jointly seek applicants for a tenured position at the level of **ASSOCIATE PROFESSOR** or **PROFESSOR** in the Department of Chemistry beginning January 1, 2005. The successful candidate must have a distinguished record of research achievement in any area of chemistry related to pharmaceutical sciences, including (but not limited to) synthetic, computational, or biophysical chemistry. Excellent oral and written communication skills are essential, as are teaching experience and relevant, high-impact publications in peer-reviewed journals. The successful candidate is expected to play a leading role in establishing a new graduate program in pharmaceutical sciences at UCI and will also advise on the development of an undergraduate major in this field. Accordingly, he or she must have, in addition to an internationally recognized research program in chemistry, a strong interest in interdisciplinary research and experience in pharmaceutical/medicinal chemistry or a related discipline. Applicants should submit a letter of application, curriculum vitae, a sample of recent scientific research papers, and a minimum of three references to:

Richard Chamberlin, Ph.D.
Chair, Search Committee, Chemistry/
Pharmaceutical Sciences
Department of Chemistry
University of California, Irvine
Irvine, CA 92697
E-mail: archambe@uci.edu

For immediate consideration, applications should be postmarked by October 15, 2004. Position will remain open until filled. Information about programs can be found at **website: http://www.chem.uci.edu.** *The University of California is an Equal Opportunity Employer committed to excellence through diversity.*

TENURE-TRACK POSITION
IMMUNOLOGY

The Department of Microbiology and Immunology at the SUNY Upstate Medical University is recruiting new faculty at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR.** Candidates should have a M.D., Ph.D., or equivalent and have sufficient experience to establish an independent, funded research program in molecular or cellular immunology. Competitive salary and startup packages are available including recently renovated laboratory and office space. Applications should be received by November 1, 2004, to receive full consideration but the search will continue until all positions are filled. Send curriculum vitae, summary of research interests, and three letters of references to: **Steven Taffet, Ph.D., Chair, Faculty Search Committee, Department of Microbiology and Immunology, SUNY Upstate Medical University, 750 E. Adams Street, Syracuse, NY 13210. E-mail: taffets@upstate.edu.**

The Upstate Medical University is an Affirmative Action/Equal Opportunity Employer.

Tenure-track **ASSISTANT/ASSOCIATE PROFESSOR** of Biology, Department of Biological Sciences, Hunter College, CUNY. Qualifications: Ph.D in molecular or cell biology, and a demonstrated commitment to research and teaching. Postdoctoral and teaching experience, publications in peer-reviewed journals, and a record of extramural funding preferred. Active, independent programs of extramurally funded research in genetics or cell biology are expected to be established or sustained as well as teaching in one or more of these areas at the undergraduate and graduate levels. Salaries and startup packages are competitive, commensurate with experience.

Send curriculum vitae, a statement of research interests, representative publications, and names of references to: **Professor Shirley Raps, Chair Search Committee, Department of Biological Sciences, Hunter College, CUNY, 695 Park Avenue, New York, NY 10021.**

POSITIONS OPEN

The University of Georgia

TWO TENURE-TRACK FACULTY POSITIONS IN GLYCOSCIENCE
The University of Georgia

Complex Carbohydrate Research Center (CCRC) is seeking candidates for two Tenure-track Faculty positions to enhance our emphasis in biomedical glycosciences. While we are particularly interested in candidates with strong backgrounds in glycosaminoglycan structure/function, glyco-immunology, cell/developmental, cancer, or chemical biology, we welcome outstanding applicants from all areas of glycobiology. Individuals who employ novel biophysical methods to address the biological functions of glycoconjugates are also invited to apply. Demonstrated creativity, commitment to teaching excellence, and willingness to participate in a multidisciplinary, collegial environment are essential. The CCRC presently has 15 tenured/tenure-track faculty members who have a common interest in the chemistry and biology of complex carbohydrates and glycoconjugates. The CCRC is home to five funded Centers: two National Center for Research Resources/NIH, one National Science Foundation, one Department of Energy, and the University of Georgia Cancer Center. Successful candidates will occupy laboratory and office space in the new 140,000 square feet CCRC building equipped with outstanding analytical, synthetic, biochemical, and cell culture/fermentation facilities. Applications received by December 1, 2004, are assured of consideration. Rank, salary, and startup funds will be commensurate with qualifications and experience. Applicants should send a cover letter, curriculum vitae, a detailed description of research goals, and arrange for four letters of recommendation to be sent to **e-mail: khoward@ccrc.uga.edu** or to: **Chair, Faculty Search Committee, Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, GA 30602-4712, U.S.A.** *An Affirmative Action/Equal Employment Opportunity Institution.*

The Department of Biology invites applications for an anticipated tenure-track position at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR** available September 2005. Qualifications include a Ph.D. in microbiology or a related discipline, substantial postdoctoral/professional experience, and evidence of outstanding research and academic potential. Candidates are expected to have or to develop an independently funded research program of national caliber and to participate in teaching. All fields of microbiology will be considered for this position. The successful candidate will complement the existing research strengths of our Department (**website: http://www.biology.neu.edu**) and will have an opportunity to participate in the interdisciplinary Biotechnology Institute.

Applicants should send curriculum vitae, a statement of research interests and plans, and a statement of teaching experience and/or interests to: **Microbiology Faculty Search Committee, 134 Mugar Hall, Northeastern University, Boston, MA 02115.** Please arrange to have at least three letters of reference sent independently to the committee. Review of applications will begin November 15, 2004, and will continue until the position is filled. *Northeastern is an Equal Opportunity/Affirmative Action Title IX University. Women and minority candidates are especially encouraged to apply.*

The University of Michigan, School of Public Health, Department of Environmental Health Sciences seeks applications for tenure-track **ASSISTANT PROFESSOR** positions in the areas of exposure assessment and environmental microbiology. Concurrent searches are underway for **DEPARTMENT CHAIR** and for an **OPEN-RANK POSITION** in risk science. Interested candidates should visit **website: http://www.sph.umich.edu/ehs.** *The University of Michigan is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

ASSISTANT PROFESSOR
Vertebrate Developmental Biology

The University of Texas at Austin (UT-Austin) invites applications for a tenure-track position as an Assistant Professor in the Section of Molecular Cell and Developmental Biology. We seek an outstanding investigator who will build an active research program addressing important research questions in vertebrate developmental biology. The successful applicant will be joining the biology community at UT-Austin during an exciting phase of growth, with recent hires in cell biology, developmental biology, neuroscience, structural biology, and related areas. Very generous startup funds are available, and the successful candidate will also be eligible for affiliation with the Institute for Cellular and Molecular Biology, which provides state-of-the-art facilities and supports an excellent graduate program.

To ensure full consideration, all application materials should be received by November 15, 2004. Applicants should send their curriculum vitae, a statement of current and future research interests, representative publications, and three letters of reference to:

Chair, Search Committee
c/o Maureen Meko
Section of Molecular Cell and
Developmental Biology
University of Texas at Austin
1 University Station A6700
BIO 311, 205W 24th Street
Austin, TX 78712-0183

Websites: http://www.biosci.utexas.edu/MCDB/ and **http://www.icmb.utexas.edu/.**

The University of Texas, Austin is an Equal Opportunity Employer. Qualified women and minorities are encouraged to apply.

LIFE SCIENCES TEACHING POSITION
at the University of Houston

The Honors College and the Department of Biology and Biochemistry at the University of Houston (UH) invite applications for an Instructional **ASSISTANT PROFESSOR** to contribute to the teaching mission of these units. This position is a twelve-month, nontenure-track appointment, with the possibility of annual renewal. The successful applicant will teach Honors sections of courses in Introductory Biology and Genetics, and will coordinate special programs for honors students in the natural sciences. The position requires an earned Doctorate in any appropriate area of the life sciences, broad knowledge of biology and biochemistry, prior teaching experience at the post-secondary level, and a commitment to high quality undergraduate teaching. Review of applications will begin October 15, 2004. Start date: January 2005 preferred. Please submit curriculum vitae, a statement outlining teaching experience and philosophy, and three letters of recommendation to: **Honors Biology Search Committee, The Honors College, 212 MD Anderson Library, University of Houston, Houston, TX 77204-2001.** *UH is an Equal Opportunity/Affirmative Action Employer. Minorities/Women/Veterans/Persons with Disabilities are encouraged to apply.*

THREE BIOLOGY FACULTY POSITIONS

The Department of Biology invites applications for three tenure-track faculty positions at the rank of **ASSISTANT/ASSOCIATE PROFESSOR.** A doctoral degree and record of research productivity are required; postdoctoral training and teaching experience are preferred. (Position 1) Microbiologist with interests in teaching microbial physiology and/or environmental microbiology preferred. (Position 2) Plant biologist with an interest in teaching plant physiology, plant taxonomy, and/or botany. (Position 3) Area of expertise is open, but an interest in developing an interdisciplinary, cross-curriculum research program is preferred. For more details and application instructions, visit us at **website: http://www.westga.edu/~biology/.**

The State University of West Georgia is committed to increasing the diversity of our faculty and strongly encourages applications from minorities and women.

THE UNIVERSITY OF SOUTH DAKOTA
SCHOOL OF MEDICINE

Chair, Department of Psychiatry/ Director, Brain Research Center

The University of South Dakota School of Medicine is seeking a person to both Chair the school's Department of Psychiatry and to direct development and implementation of a Brain Research Center. The School of Medicine is a community-based medical school with multiple campuses whose mission is to produce primary care physicians for South Dakota. Department faculty are primarily located and employed by a single affiliated hospital in Sioux Falls, South Dakota, where the Adult and Child and Adolescent Psychiatry Residency Programs are located. Responsibility for medical student education and community service extend across the entire state. A free-standing, 92-bed academic psychiatric hospital is scheduled for completion in 2006. The Department Chair must have Board Certification in Psychiatry, academic qualifications for appointment at Associate or Full Professor, and demonstrated skills in research, education, and administration. Excellent leadership and interpersonal skills are necessary. Priority will be given to candidates with a proven track record in nationally funded neuro-psychiatric research, as well as experience in the education of medical students and residents. Applicants must have experience in the clinical practice of Psychiatry and be licensable in South Dakota.

Applicants must send a curriculum vitae and names and addresses of three references to: **Office of the Dean, Psychiatry Search Committee, 1400 W. 22nd St., Sioux Falls, SD 57105.** Applications will be reviewed until the position is filled.

The University of South Dakota School of Medicine is an Equal Opportunity/Affirmative Action Employer committed to increasing the diversity of its faculty, staff and students.

the university of south dakota.
SCHOOL OF MEDICINE & HEALTH SCIENCES

Southwestern University
AT GEORGETOWN, TEXAS

Lillian Nelson Pratt Endowed Chair

The Department of Biology at Southwestern University invites applications from broadly trained **Evolutionary Biologists** for the Lillian Nelson Pratt Endowed Chair in Biology. Preference will be given to applicants with expertise in developmental and/or molecular approaches. Appointment will be at a rank commensurate with the experience of the individual filling the position, to begin August 2005. A PhD in a relevant discipline, a strong commitment to undergraduate teaching, and a record of distinction in both teaching and research are required.

Teaching responsibilities will include upper-level Evolutionary Biology for majors, participation in the Introductory Biology sequence, and possible additional courses as required by Departmental needs. The successful candidate may elect to participate in Southwestern's interdisciplinary Environmental Studies Program. The successful candidate will be expected to maintain a research program that actively involves undergraduates.

Southwestern University is a selective, undergraduate institution committed to a broad-based liberal arts, sciences, and fine arts education. Southwestern currently enrolls approximately 1,250 students and maintains a student to faculty ratio of 10 to 1. The University's endowment ranks among the highest per student of undergraduate institutions in the country. In addition to a number of other national organizations, Southwestern University is a member of two consortia of liberal arts colleges, the Associated Colleges of the South and the Annapolis Group. Located in Georgetown, Texas, 28 miles north of downtown Austin, Southwestern is affiliated with The United Methodist Church. Southwestern University is committed to fostering a diverse educational environment and encourages applications from members of groups traditionally under-represented in academia. For information concerning the University, visit our web site at **www.southwestern.edu.**

Interested persons should send a letter of interest, curriculum vitae, statements of teaching and research philosophies, graduate and undergraduate transcripts (unofficial), and three current letters of recommendation to: **Kendra Clovis, Faculty Secretary - Biology Search, Southwestern University, P.O. Box 770, Georgetown, TX, 78627-0770.**

Applications received by **October 22, 2004** will receive full consideration. For more information, contact the **Biology Department Chair, Dr. Rebecca Sheller, shellerr@southwestern.edu.**

Southwestern University is an Equal Opportunity Employer. EOE/M/F

USDA ARS Agricultural Research Service
www.ars.usda.gov

RESEARCH GENETICIST or RESEARCH MOLECULAR BIOLOGIST
GS-13/14/15
Salary Range of $72,454 to $130,930 per annum

The Western Human Nutrition Research Center at the University of California, Davis, invites applications for a permanent, full-time Research Scientist to study the role of genetics in dietary, behavioral and other factors that regulate energy balance, the metabolism of related nutrients, and/or obesity co-morbidities such as diabetes or cancer. The candidate is expected to establish a strong research program, as a member of a team that investigates the nutritional, biological, genetic and environmental factors that contribute to achieving and maintaining a healthy body weight and reducing risk of chronic disease. The position offers outstanding opportunities for collaborations within the WHNRC and at UC Davis, in human nutrition intervention and community studies.

A PhD or equivalent degree in genetics, molecular biology, nutrition, physiology, biochemistry or another biological science that includes training in genetics and molecular biology is desired. Demonstrated relevant expertise in studies with humans, non-human primates, or other models, and research related to human nutrition, obesity and its co-morbidities is desirable. U.S. citizenship is required. For details and application directions see: **http:www.afm.ars.usda.gov/divisions/hrd/vacancy/resjobs/0357**. Specific questions may be directed to **Dr. Lindsay Allen** at **530 752-5268** or **Dr. Nancy Keim** at **530 752-4163**.

For a printed copy by mail call **301-504-1482**. Announcement closes **December 3, 2004**.

USDA/ARS is an Equal Opportunity Employer and Provider.

McLaughlin Research Institute for Biomedical Sciences

Faculty Position in Mouse Genetics/Genomics

McLaughlin Research Institute has opened a search for an innovative mammalian geneticist. Candidates should possess a doctoral degree and a record of research excellence as a postdoctoral fellow or independent investigator. The applicant should have, or be capable of developing, a productive independent research program that can compete successfully for grant funding. Applicants with interests in animal models for human disease, stem cell biology and differentiation, or novel strategies for mutant production or genome modification are particularly encouraged to apply. Candidates should be able to establish both intramural and extramural collaborative projects.

McLaughlin Research Institute offers a unique opportunity for research programs with an emphasis in mammalian genetics. The Institute is a small non-profit organization and offers a non-bureaucratic, interactive research environment. The Institute is housed in a spacious modern research building with an excellent mouse facility that includes a transgenic and gene-targeting service.

For additional information see **www.montana.edu/wwwmri**. For specific questions about the Institute contact **George Carlson, Pin-Xian Xu, John Mercer,** or **John Bermingham** at MRI, or any of the following members of our Scientific Advisory Committee: **Irv Weissman, David Baltimore, David Cameron, Neal Copeland, Jeff Frelinger, Leroy Hood, Nancy Jenkins, or James Spudich**.

Applications, including names of individuals we may contact for references, should be sent to:

George A. Carlson, Ph.D.
Director, McLaughlin Research Institute
1520 23rd Street South
Great Falls, MT 59405

An Equal Opportunity/Affirmative Action Employer

PHYTOCHEMIST

The Department of Plant Biology at Southern Illinois University, Carbondale (SIUC) has an opening for a tenure-track **ASSISTANT PROFESSOR** working in any area of plant biochemistry, secondary metabolism, phytopharmacognosy, or medicinal compounds/nutraceuticals. Research should focus on model or non-agricultural plants, or utilize a systems biology approach. Demonstrated experience with modern separation techniques is desired. Startup includes a new high-performance liquid chromatography and gas chromatography. A Ph.D. in a relevant discipline is required. Applicants must have a record of peer-reviewed publications. Postdoctoral experience and/or past evidence of external funding are preferred. Candidates are expected to develop an externally funded research program. The successful applicant will teach at the undergraduate level and offer graduate courses in the candidate's field of expertise. Candidates should send a letter of application, curriculum vitae, representative publications, three letters of reference, and statements of research goals and teaching philosophy to: **Dr. Stephen Ebbs (e-mail: sebbs@plant.siu.edu) Search Committee Chair, Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901-6509.** Department and University information can be found at **website: http://www.science.siu.edu/plant-biology/.** Review of applications begins October 11, 2004, and will continue until the position is filled. *SIUC is an Affirmative Action/Equal Opportunity Employer that strives to enhance its ability to develop a diverse faculty and staff, and to increase its potential to serve a diverse student population. All applicants are welcome and encouraged and will receive consideration.*

FACULTY POSITIONS, BIOLOGICAL CHEMISTRY
University of Massachusetts, Amherst
Department of Chemistry

Applications are invited for two tenure-track faculty positions in the areas broadly defined as experimental biological chemistry and biomaterials to begin in September 2005. Individuals are sought who will develop research programs at the interface of chemistry and biology. Successful applicants will apply chemical approaches to current problems in biology and/or biological approaches to current problems in chemistry, in areas that are complementary to and synergistic with an interdisciplinary and interdepartmental group of faculty (visit **website: http://www.chem.umass.edu/** for details). The search targets the **ASSISTANT PROFESSOR** level, although outstanding applicants for higher ranks will be considered. Salary will be commensurate with qualifications and experience. Evaluation of applications will begin on November 1, 2004, and continue until the position is filled. Candidates should submit curriculum vitae, statements of research and teaching plans, and arrange to have three letters of recommendation sent to: **Faculty Search Chair, Department of Chemistry, 701 Lederle Graduate Research Tower, University of Massachusetts, 710 North Pleasant Street, Amherst, MA 01003-9336.** *The University of Massachusetts is an Affirmative Action/Equal Opportunity Employer.*

The Department of Ecology and Evolutionary Biology, Tulane University, invites applications for two tenure-track positions, one in evolutionary systematics and one in plant ecology, at the level of **ASSISTANT PROFESSOR.** See **website: http://www.tulane.edu/~eeob/faculty_search.html** for details on each position. Send curriculum vitae, statements of research and teaching interests, selected publications, and names and addresses of three references to: either **Evolutionary Systematist Search** or **Plant Ecologist Search** at the **Department of Ecology and Evolutionary Biology, 310 Dinwiddie Hall, Tulane University, New Orleans, LA 70118-5698.** Review of applications will begin October 14, 2004, and the searches will remain open until the positions are filled. *Tulane University is an Affirmative Action/Equal Employment Opportunity Employer.*

POSITIONS OPEN

Virginia Tech

DEPARTMENT HEAD
Plant Pathology, Physiology, and Weed Science

The College of Agriculture and Life Sciences invites applications for the position of Professor and Head of the Department of Plant Pathology, Physiology, and Weed Science. The Department has 26 faculty members, with outstanding research programs in molecular biology, functional genomics, and basic biology of plant-microbe interactions, plant-plant interactions, abiotic stress physiology, metabolic engineering, conventional plant protection approaches, and weed biology/management. A full position description and additional information on departmental programs is available at **website: http://www.ppws.vt.edu.** Applicants should complete a faculty application and submit complete curriculum vitae, a statement of intent, and names, addresses, and telephone numbers of five references online at **website: http://jobs.vt.edu.** Review will begin on October 1, 2004, and continue until the position is filled. Additional inquiries should be directed to: **Mr. Don Ball, Fralin Biotechnology Center, Virginia Tech,** at **e-mail: biotech@vt.edu** or by **telephone: 540-231-6934.**

Equal Opportunity/Affirmative Action Employer.

The Vollum Institute is seeking an outstanding scientist for a **FACULTY APPOINTMENT**. We are particularly interested in individuals with a research focus in the general areas of molecular and cellular neuroscience, molecular genetics, and/or mechanisms of signal transduction. Although we would like to fill this position at the **ASSISTANT PROFESSOR** level, applications at more advanced levels will also be considered. We offer an attractive startup package and the opportunity to work in an outstanding scientific environment that includes strong ties to related departments at Oregon Health & Science University. Applicants should have a strong record of research, the potential to develop an independent, funded research program, and an interest in training graduate students. Candidates with a Ph.D. and/or M.D. and several years of postdoctoral experience should apply by November 15, 2004, sending a copy of their curriculum vitae, a description of research plans and goals, and the names of three references to:

Vollum Faculty Search Committee
c/o Richard H. Goodman, M.D., Ph.D.
Vollum Institute, L474
Oregon Health & Science University
3181 SW Sam Jackson Park Road
Portland, OR 97201-3098
Website: http://www.ohsu.edu/vollum

The Vollum Institute is an Equal Opportunity/Affirmative Action Employer committed to maintaining diversity in its faculty.

CHEMICAL OR BIOCHEMICAL ENGINEER

ProMetic BioSciences is a leading international biopharmaceutical company specializing in research, development, manufacture, and commercialization of products and applications for the biopharmaceutical industry. The Research and Development Department of ProMetic BioSciences is searching for a Chemical or Biochemical Engineer to work on sponsored projects in laboratories in the Chemical Engineering Department at North Carolina State University. Candidates should have a Ph.D. preferably with one-to-three years experience in process development and device design, particularly on membranes and chromatography, for the purification, detection, and removal of proteins and/or pathogens such as prion protein, viruses, and toxins. Forward curriculum vitae to: **Professor Ruben Carbonell, Chemical Engineering Department, North Carolina State University, Campus Box 7006, Raleigh, NC 27695-7006. Fax: 919-515-5831. E-mail: ruben@ncsu.edu.** ProMetic **website: http://www.prometic.com.**

POSITIONS OPEN

CYTOGENETICS POSITION
Department of Pathology
Northwestern University
Feinberg School of Medicine

Medical Director of Cytogenetics. Applications are invited for a full-time, continuing tenure-track appointment at the rank of **ASSOCIATE PROFESSOR/PROFESSOR** level in the Department of Pathology, Northwestern University, Feinberg School of Medicine, Chicago, to serve as Medical Director of the Cytogenetics Program, which will include prenatal and tumor cytogenetics at the Northwestern Memorial Hospital. This individual should hold M.D., or M.D./Ph.D. or Ph.D. degree(s) and be board certified/eligible in clinical pathology, and/or medical cytogenetics, as well as eligible for an unrestricted medical license in the state of Illinois. Full service laboratories in diagnostic molecular pathology, molecular microbiology, FISH Laboratory, diagnostic bacteriology, virology, mycobacteriology, mycology, and parasitology are located in a superb physical facility. Direction of these laboratories includes responsibility for College of American Pathologists accreditation. This individual is expected to establish a vigorous independent and interactive research program in basic and/or translational research areas and required participation in the training and education of residents and fellows. Salary and rank will be commensurate with experience. The start date for this position will be September 1, 2005. Please submit your curriculum vitae, a statement of areas of interest, expertise and strengths, and names of three references to: **Janardan K. Reddy, M.D., Chair, Department of Pathology, Northwestern University, Feinberg School of Medicine, 303 East Chicago Avenue, Chicago, IL 60611,** or to: **Nancy Starks** via **e-mail: nstarks@northwestern.edu** no later than November 30, 2004.

Northwestern University is an Affirmative Action/Equal Opportunity Employer. Hiring is contingent upon eligibility to work in the United States. Women and minorities are encouraged to apply.

TENURE-TRACK FACULTY POSITIONS
Northwestern University

The Department of Biochemistry, Molecular Biology, and Cell Biology (BMBCB) seeks two outstanding scientists whose research addresses how individual molecules combine to form complex structures that carry out the work of the cell. We are particularly interested in work on the biochemical and biophysical aspects of molecular machines or the cell biology of complex assemblies. The successful candidate will join a vibrant interdisciplinary community working on related problems. Northwestern is expanding the life sciences and has recently opened a new building, the Pancoe/ENH Pavilion (**website: http://www.biochem.northwestern.edu**). Applicants should submit a cover letter, curriculum vitae, research summary and statement of future research goals, and statement of teaching experience and interests, and arrange for four letters of recommendation to be sent on their behalf. Materials should be submitted electronically as Adobe Acrobat or Microsoft Word files to **e-mail: bmbcb@northwestern.edu** using "BMBCB Search" as the subject. Mail correspondence may be sent to: **Faculty Search Committee, BMBCB, 2205 Tech Drive, Northwestern University, Evanston, IL 60208-3500.** Review will commence immediately and remain active until the positions are filled.

Affirmative Action/Equal Opportunity Employer. Women and minorities are especially encouraged to apply.

The Department of Biology at the University of Colorado at Colorado Springs (UCCS) invites applications for a tenure-track **ASSISTANT PROFESSOR** position in biochemistry. Review of applications will begin in October 2004. The academic appointment begins August 2004. For details go to **website: http://www.uccs.edu/~biology/Search.htm.**

The University of Colorado is an Equal Opportunity/Affirmative Action Employer and encourages a diversity of applicants.

PENNSTATE

University Park

Faculty Position in Microbiology

We seek an outstanding individual who uses contemporary molecular approaches to investigate important processes in any area of microbial gene regulation, biochemistry or physiology. Candidates employing systems-level approaches are especially encouraged to apply.

Faculty Position in Gene Regulation

We seek an outstanding individual who uses biochemical, genetic, cytological, and/or computational approaches to investigate any area of eukaryotic gene regulation or nuclear function.

Applications for tenure-track positions will be considered at all ranks (**ASSISTANT, ASSOCIATE,** or **FULL professor**). Successful candidates will be expected to participate in undergraduate and graduate education, and to develop a strong externally funded research program. Applications should be submitted electronically in PDF format to **L-bmbfacsearch @lists.psu.edu** or in print to the relevant **Microbiology or Gene Regulation Search Committee, Dept. of Biochemistry and Molecular Biology, 108 Althouse Laboratory, The Pennsylvania State University, University Park, PA 16802.**

Applications should include the following as separate files: (1) a cover letter specifying the position and rank of interest, (2) curriculum vitae (including funding history), (3) a summary of your research accomplishments, future research plans, and teaching interests, (4) up to three reprints that reflect your work, and (5) three letters of reference sent under separate cover (applicable to Assistant rank only). Review of applications will begin on **October 15, 2004**.

The University Park campus is Penn State's main campus, located in central Pennsylvania and enrolling over 40,000 students. Penn State is a member of the Big Ten affiliation of universities.

Penn State is committed to Affirmative Action/Equal Opportunity and the diversity of its workforce.

PENNSTATE

University Park

Faculty Positions in Macromolecular Crystallography

The Huck Institutes of the Life Sciences, the Department of Chemistry, and the Department of Biochemistry and Molecular Biology at Penn State invite applications for two tenured/tenure-track faculty positions at Assistant, Associate or Full Professor level in X–ray crystallography. We are particularly interested in macromolecular crystallographers possessing research excellence and interests in mechanistic enzymology, membrane proteins, RNA and RNA-protein interactions, or hormone receptors to build on existing strengths at Penn State.

Applications should include current curriculum vitae, a summary of past research experience, and a statement of future research goals. In addition, three letters of reference for applicants at Assistant Professor level should be sent to:

Chair, Crystallography Search, Box SS
Dept. of Biochemistry and Molecular Biology
108 Althouse Laboratory
The Pennsylvania State University
University Park, PA 16802

Applications will be reviewed as they are received. Completed applications received by **November 26, 2004,** will receive priority.

Penn State is committed to Affirmative Action/Equal Opportunity and the diversity of its workforce.

CCSG Administrator

The University of Texas M. D. Anderson Cancer Center (MDACC) is seeking a talented individual to provide administrative coordination for its comprehensive Cancer Center Support Grant (CCSG). Currently, MDACC holds the largest number of NCI grants and the largest amount of NCI funding among academic institutions. The CCSG grant supports 19 programs and 19 shared resources. Working with the PI of the CCSG, President of MDACC, and the Co-PI of the CCSG, Vice President for Translational Research, the CCSG Administrator will coordinate, analyze, and support all aspects of our Cancer Center Support Grant. Other duties include developing databases, preparing budgets, initiating grant renewals, writing grants, and coordinating all financial and reporting activities. To qualify for this key position, you must possess a degree, preferably a Master's or Ph.D.in Biology, Chemistry, Business or Health Administration. The successful candidate must also have had at least five years' experience in preparing grants, developing budgets, and managing resources.

Qualified candidates may contact: Dr. Robert Bast Jr., E-mail: rbast@mdanderson.org; Ph: 713-792-7743.

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER
Making Cancer History®

M. D. Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status except where such distinction is required by law. All positions at the University of Texas M. D. Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free environment.

Department of Geosciences
PRINCETON UNIVERSITY

The Department of Geosciences at Princeton University is seeking applications for a new tenure-track faculty position in the areas of solid-earth **geophysics** or **geochemistry**. We anticipate hiring at the assistant professor level, although candidates at a higher rank may be considered under exceptional circumstances. We are particularly interested in outstanding individuals who will interact with and complement existing research programs in global seismology, mineral physics, the physics of earthquakes, and structural geology (see our website at **http://geoweb.princeton.edu**). Examples of appropriate research areas include, but are not limited to, computational dynamics of the mantle and/or lithosphere, crustal deformation and active tectonics, physical or chemical evolution of the Earth or terrestrial planets, mantle petrology, earthquake seismology, and mineral or rock physics.

Applicants should send a curriculum vitae, including a publication list, a statement of research and teaching interests, and contact information for three references to: **Search Committee, Department of Geosciences, Guyot Hall, Princeton University, Princeton, NJ 08544. The starting date is flexible, ranging up to September 2006**. Evaluation of applications will begin immediately; interviews of candidates will begin in September 2004 and continue until the position is filled.

For general information about applying to Princeton and how to self-identify, please link to **http://web.princeton.edu/sites/dof/ApplicantsInfo.htm**.

Princeton University is an Affirmative Action Equal Opportunity Employer; women and members of minority groups are encouraged to apply.

FACULTY OPENING IN EXPERIMENTAL BIOLOGICAL PHYSICS
Washington University in St. Louis

The Department of Physics invites applications for a tenure-track appointment in experimental biophysics at the **ASSISTANT PROFESSOR** level, to begin fall 2005.

We seek individuals with an outstanding research record and independent creativity in applying experimental tools combined with quantitative models to study living systems at an integrated level. Applicants should have a strong background in physics and an aptitude for teaching and mentoring both undergraduate and graduate students. The successful candidate will complement and reinforce the Department's strength in biologically oriented physics and may take advantage of the top-ranked Washington University Medical School and the vigorously growing Department of Biomedical Engineering. Applications will be considered until the position is filled, but priority will be given to those received by November 15, 2004. Applicants should send their curriculum vitae with a publication list and a statement of research interests and future plans, and ask three references to send letters of evaluation. Correspondence should be sent to: **Professor John W. Clark, Biological Physics Search, Department of Physics, CB 1105, Washington University, 1 Brookings Drive, St. Louis, MO 63130-4899** or by **e-mail: jwc@wustl.edu.** *Washington University is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.*

The Division of Cardiovascular Medicine at the Brigham and Women's Hospital, directed by **Peter Libby, M.D.,** invite applications for a faculty position at the level of **INSTRUCTOR/ASSISTANT PROFESSOR.** Candidates should have an M.D., Ph.D., or equivalent degree with a strong interest in basic research in the areas of cardiac development and/or regeneration. It is anticipated that the candidate will develop an independent research and secure extramural funding. Academic rank and salary will be commensurate with experience. The candidate will join a newly established Program in Cardiovascular Transcriptional Biology under the direction of **Mukesh K. Jain, M.D.**

Applicants should send their curriculum vitae, summary of present and future research plans, and names of three references to:

Mukesh K. Jain, M.D.
Chair, Search Committee
Cardiovascular Division
Brigham and Women's Hospital
Thorn Research Building, Room 1127
20 Shattuck Street
Boston, MA 02115
E-mail: mjain@rics.bwh.harvard.edu

The Brigham and Women's Hospital is an Equal Opportunity Employer, and encourages applications from female and minority candidates.

The Division of Pediatric Pulmonology at CWRU, Rainbow Babies and Children's Hospital at Case Western Reserve University (CWRU) is seeking a **PHYSICIAN-SCIENTIST** interested in conducting basic science and/or translational research in asthma. The Division operates a large children's asthma center and is actively involved in a number of federally funded clinical trials. Areas of active research based in the Division include cell signaling, airway and inflammatory cell biology, and the genetics of inherited respiratory diseases. The Division currently includes 10 full-time M.D.s and seven Ph.D.s; ample laboratory space and resources, as well as protected time, are available. Applicants should be board certified/board eligible in pediatric pulmonology or allergy. Please send inquiries or applications electronically to: **Carolyn M. Kercsmar, M.D. (e-mail: cxk8@cwru.edu**). Applications should include full curriculum vitae, statement of goals, and names of three references. *CWRU is an Affirmative Action/Equal Opportunity Employer and women and minority applicants are encouraged to apply.*

POSITIONS OPEN

PROTEIN MASS SPECTROMETRY

Dartmouth Medical School and the Norris Cotton Cancer Center invite applications for a tenure-track faculty position at the level of **ASSISTANT** or **ASSOCIATE PROFESSOR.** The ideal candidate is an innovative, interactive protein mass spectrometrist with a strong research program. Qualified individuals should submit a letter of application with a statement of career goals and curriculum vitae to: **Dr. Charles Brenner, Norris Cotton Cancer Center, One Medical Center Drive—Rubin 733, Lebanon, NH 03756.** Three letters of recommendation should be sent to the same address. *Dartmouth is an Equal Opportunity/Affirmative Action Employer and encourages women and minority candidates to apply.*

ASSISTANT/ASSOCIATE PROFESSOR
Cell/Developmental Biology
Eckerd College

Full-time, tenure-track position beginning fall 2005. Teaching responsibilities include introductory courses in cell/molecular biology and genetics and an upper-level course in developmental biology. Demonstrated interest and potential in undergraduate teaching are essential. The successful candidate should establish a research program involving undergraduate students and will contribute to anticipated revision of the biology program. Participation in an interdisciplinary, values-oriented general education program is expected, including a regular rotation in a two-semester freshman course that explores western and non-western traditions. Send letter of application including statements of teaching and research interests, curriculum vitae, undergraduate and graduate transcripts, and three letters of reference by October 30, 2004, to: **Dr. Steven H. Denison, Natural Sciences, Eckerd College, 4200 54th Avenue South, St. Petersburg, FL 33711. Telephone: 727-864-8456; fax: 727-864-8382; e-mail: denisosh@eckerd.edu.** Eckerd College is an outstanding college of the liberal arts and sciences with strong programs in biology and marine biology and is located on Tampa Bay in St. Petersburg, Florida. Candidates with research interests in marine organisms are encouraged to apply. No electronic applications will be accepted. *Equal Opportunity Employer/Minorities/Females/Veterans/ADA.*

NEUROSCIENCE FACULTY POSITION
The University of North Carolina at Chapel Hill

The Department of Cell and Molecular Physiology in the School of Medicine invites applications for a tenure-track faculty position (rank dependent on qualifications). Applicants must be at least two years postdoctoral. We seek candidates using novel approaches to study the physiology or pathophysiology of the nervous system. Preference will be given to individuals with established record of exciting, contemporary work on integrative neural function. An attractive startup package and new laboratory space are available. The Department's faculty are expected to contribute to teaching of graduate and medical students.

Please submit the names of four potential references, curriculum vitae, statement of the proposed research program, and career goals by e-mail to:

Neuroscience Search Chair
Edward R. Perl
Department of Cell and Molecular Physiology
5109 Neuroscience Research Building CB #7545
School of Medicine
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27599-7545
E-mail: neurosearch@med.unc.edu

Closing date for first consideration: October 11, 2004. *UNC is an Equal Opportunity Employer.*

POSITIONS OPEN

FACULTY POSITION
The Fred Hutchinson Cancer Research Center

The Division of Human Biology at the Fred Hutchinson Cancer Research Center is soliciting applications to fill an open faculty position in the broad areas of genomics and genetics. Applicants with outstanding accomplishments, particularly in the areas of complex disease-gene mapping, population genetics, or large-scale approaches to functional genomics, proteomics, or bioinformatics are encouraged to apply.

The Human Biology Division fosters interdisciplinary, collaborative research at the interfaces of basic, clinical, and population sciences in order to further our understanding of human biology, cancer, and other complex human diseases.

The Division occupies state-of-the-art research laboratories on a new lakeside campus. The Center offers outstanding shared resources, including DNA array, sequencing, and proteomics facilities. The Center has active graduate-student and postdoctoral training programs and offers exceptional opportunities for scientific interactions with other investigators in the Seattle area.

Additional information about the Division can be found at **website: http://www.fhcrc.org/science/human_biology/.**

Candidates should send curriculum vitae, a concise statement of research plans, and three letters of reference to: **Human Biology Faculty Search Committee, Fred Hutchinson Cancer Research Center, Division of Human Biology, Mailstop: C3-168, 1100 Fairview Avenue North, P.O. Box 19024, Seattle, WA 98109-1024.** The application deadline is October 15, 2004.

The Fred Hutchinson Cancer Research Center is an Equal Opportunity Employer committed to work force diversity. Applications from female and minority candidates are strongly encouraged.

WATER RESOURCE SCIENTIST
Baylor University

Baylor University seeks a candidate with skills in water resource modeling and/or aquatic chemistry for a tenure-track faculty position, **ASSISTANT PROFESSOR** level, beginning August 2005. Ph.D. or equivalent is required. Position responsibilities: teach at graduate and undergraduate levels and establish a vigorous, externally funded research program. Will participate in the new interdisciplinary Ph.D. program in ecology and environmental sciences and in the Center for Reservoir and Aquatic Systems Research (**website: http://www.baylor.edu/crasr**). Applications will be reviewed beginning October 15, 2004, and will be accepted until the position is filled. To ensure full consideration, your application must be completed by November 5, 2004. Send application letter, statements of research interests and teaching philosophy, curriculum vitae, reprints of up to three publications, graduate and undergraduate transcripts, representative teaching evaluations, if available, and contact information for three references to: **Search Committee Chair, Department of Environmental Studies, One Bear Place #97266, Baylor University, Waco, TX 76798-7266. Telephone: 254-710-3405; fax: 254-710-3409; e-mail: bryan_brooks@baylor.edu; website: http://www.baylor.edu/Environmental_Studies/.** Baylor is a Baptist university affiliated with the Baptist General Convention of Texas. *As an Affirmative Action/Equal Employment Opportunity Employer, Baylor encourages minorities, women, veterans, and persons with disabilities to apply.*

ASSISTANT PROFESSOR OF BIOLOGY. Saint Vincent College invites applications for a tenure-track position beginning fall 2005. Requirements include a Ph.D. and commitment to undergraduate education in a liberal arts setting. Postdoctoral experience is preferred. Teaching responsibilities include general biology, comparative vertebrate anatomy, a course for nonscience majors, either developmental biology or histology, and supervision of required senior research projects. Please see details and application procedures at **website: http://www.stvincent.edu/services/hr/posting.jsp.** *Equal Opportunity Employer/Affirmative Action.*

PHYSIOLOGY UCLA

Assistant or Associate Professors (2)

The **Department of Physiology** at the **David Geffen School of Medicine at UCLA** invites applications for tenure-track faculty positions, preferably at the level of Assistant or Associate Professor.

We are especially interested in candidates in molecular physiology with interests in functional genomics, proteomics, or transgenics. Areas of departmental strength include molecular biophysics and cardiovascular research, and candidates in these disciplines are encouraged to apply. However, we will consider applicants in all areas of modern physiology. Areas in which we might hope to expand include renal and respiratory physiology. Candidates are expected to have a strong background in cellular and molecular biology and a demonstrated interest in the study of physiological systems in model organisms.

The successful candidate will be expected to develop an independent research program and participate in the teaching mission of the Department for medical, dental, and graduate students.

Interested applicants should e-mail their curriculum vitae, a letter with a statement of research interests and career goals to **Dr. Thomas O'Dell** at **PhysiologySearch@mednet.ucla.edu**. Applicants should also arrange for three letters of reference to be sent to **Dr. O'Dell** at the same e-mail address.

UCLA is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.

James P. Wilmot Cancer Center
University of Rochester

Human Cancer Biology

The Wilmot Cancer Center and the Department of Biomedical Genetics at the University of Rochester Medical Center invite applications from candidates interested in the biology of solid tumors. The Medical Center offers exciting opportunities for interdisciplinary/translational cancer research in an interactive environment with strong programs in cancer and stem cell biology, cell signaling, genetics in model organisms, genomics, proteomics and bio-mathematics. This is a tenure-track position at the Assistant/Associate Professor level. Candidates should have a strong record of accomplishment and evidence of extramural funding.

To apply, submit *curriculum vitae*, statement of research interests/plans, and the names of three referees via e-mail to:

Dr. Hartmut Land
Chair, Biomedical Genetics
Director, Basic Sciences, J.P. Wilmot Cancer Center
E-mail: cancerbio@urmc.rochester.edu

See: **http://www.urmc.rochester.edu/Aab/bg/**
http://www.stronghealth.com/services/cancer

The University of Rochester is an Equal Opportunity Employer.

NYU Medical Center — We enhance medicine.
SCHOOL OF MEDICINE — NEW YORK UNIVERSITY
NYU**Cancer**Institute

The Cancer Institute of the NYU School of Medicine, under the direction of Dr. Steven Burakoff, announces an unprecedented expansion in its programs in cancer biology at the new Joan and Joel Smilow Research Building.

Faculty Recruitment in Basic and Translational Research

We are seeking up to ten new tenure track faculty recruits engaged in basic and/or translational research related to cancer. All will be members of the NYU Cancer Institute with primary academic appointments in one of the basic science or clinical departments. Laboratories will be available in the Smilow Research Building, currently under construction. Most positions will be at the Assistant Professor level, although a few Associate or Professor level recruitments will be considered. Areas of interest include, but are not limited to, **Animal Models of Cancer, Cancer Immunology, Cancer Neurobiology, DNA damage and Checkpoint Control, Molecular Carcinogenesis, Molecular Control of Cell Proliferation and Stem Cell Biology.** Investigators whose research will enhance the translation of basic research findings into new therapeutic interventions and the design of new clinical trials are encouraged to apply. Successful applicants should hold an MD and/or PhD with established reputations in these areas of research and visibility at the national level.

Interested investigators are encouraged to visit **http://www.med.nyu.edu/smilowcenter** to learn more about the NYU Cancer Institute, qualifications for candidates and our recruitment process. Qualified candidates can apply by following the instructions found on the web site. We are an equal opportunity employer and provide a drug-free workplace.

MAYO CLINIC

Physician Scientist Faculty Position

Rochester, Minnesota

The Department of Medicine at Mayo Clinic in Rochester, MN invites applications from persons interested in conducting patient or disease oriented research in support of cross-disciplinary research programs. Successful applicants will have an established extramurally funded research program and will receive substantial intramural support. Areas of research focus of particular interest include Aging, Clinical Immunology and Immunotherapeutics, Complementary and Integrative Medicine, Translational Immunovirology and Biodefense, Cancer or Molecular Medicine.

The successful candidate will have an MD, Ph.D. or an MD/Ph.D. degree(s) and must be eligible for hire at Mayo.

To learn more about Mayo Clinic and Rochester, MN, please visit **www.mayoclinic.org**

Interested applicants should submit a curriculum vitae, a statement of research interests and goals, along with the name of three references by November 1, 2004, to:

Vicente Torres, MD
Chair, Search Committee
Eisenberg Building S24

Mayo Clinic College of Medicine
200 First Street SW
Rochester, MN 55905

Mayo Foundation is an affirmative action and equal opportunity educator and employer. Post offer/pre-employment drug screening is required.

FACULTY POSITIONS IN CHEMISTRY AND BIOCHEMISTRY
University of California Santa Barbara

The Department of Chemistry and Biochemistry at the University of California Santa Barbara announces a search for a tenure-track faculty member to begin in fall 2005 at the **ASSISTANT PROFESSOR** level. Outstanding candidates with research and teaching interests in all sub-areas of inorganic chemistry including bioinorganic, inorganic materials, and organometallic chemistry are invited to apply. In addition to contributing to the research, teaching, and service missions of the Department of Chemistry and Biochemistry, we anticipate campus-wide interactions in interdisciplinary programs between departments in the Colleges of Letters and Sciences and Engineering, along with other University-based Centers and Institutes. Applicants should submit curriculum vitae, description of their research plans, graduate and undergraduate teaching interests, and arrange to have three letters of recommendation sent on their behalf to: **Faculty Search Committee, Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, CA 93106-9510.** Review of applications will begin October 1, 2004, and will continue until the position is filled. A Ph.D. is required at the time of appointment. The Department is especially interested in candidates who can contribute to the diversity and excellence of the academic community through research, teaching, and service. *The University of California is an Equal Opportunity/Affirmative Action Employer.*

ANIMAL ECOLOGY
University of Wyoming

The Department of Zoology and Physiology at the University of Wyoming invites applications for a tenure-track position in animal ecology beginning August 2005 at the level of **ASSISTANT PROFESSOR**, or at a higher rank for an individual with an outstanding research and funding record. Ph.D. required for faculty rank. Teaching responsibilities include an introductory course in ecology or fisheries and wildlife biology plus an upper-division course in the candidate's area of expertise. Research interests can involve any aspect of animal ecology although a focus on spatial aspects of population dynamics and habitat use would complement existing areas of expertise in our Department. The candidate will be expected to develop an extramurally funded research program, advise undergraduates in our wildlife and fisheries biology major, and mentor graduate students. The candidate will be encouraged to interact with natural resource agencies and with the University of Wyoming's School of Environment and Natural Resources.

Review of applications will begin on November 1, 2004. Applicants should send curriculum vitae, statements of research and teaching interests, and arrange to have three letters of reference sent to: **Chair, Animal Ecology Search Committee, Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071-3166. Website: http://uwadmnweb.uwyo.edu/Zoology.** *The University of Wyoming is an Affirmative Action/Equal Opportunity Employer.*

CAREER IN OPTOMETRY, OPTOMETRIC RESEARCH, OR TEACHING

The New England College of Optometry offers a unique program for those with a Doctorate in the sciences: biology, chemistry, physics, psychology, pharmacology, medicine, etc. Candidates have the opportunity to obtain the Doctor of Optometry (O.D.) degree in 27 months. The program begins annually in early March. Employment opportunities exist in clinical practice, industry, optometric faculty positions, and research. **Contact: the Admissions Office, Department S, 424 Beacon Street, Boston, MA 02115. Telephone: 1-800-824-5526; e-mail: admissions@ne-optometry.edu. Website: http://www.ne-optometry.edu.** Application deadline: February 1, 2005.

POSITIONS OPEN

NICHOLLS STATE UNIVERSITY

Nicholls State University (NSU) invites applications for the position of **ASSISTANT PROFESSOR** of molecular biology in the Department of Biological Sciences. Visit **website: http://www.nicholls.edu/hr** for complete position announcement and application instructions. *NSU is an Affirmative Action/Equal Opportunity Employer.*

TENURE-TRACK FACULTY POSITION IN MICROBIOLOGY
Indiana University, Bloomington

The Indiana University (IU) Department of Biology (**website: http://www.bio.indiana.edu**) and the Interdisciplinary Human Biology Program invite applications for a Tenure-track Faculty position in bacterial cell and molecular biology, with emphasis on pathogenesis, including cellular microbiology. This position is part of a significant, continuing expansion in the life sciences at IU Bloomington and represents an exceptional opportunity to join a strong Microbiology Program in the Department of Biology and new interdisciplinary initiatives in human biology and biotechnology linking together biology, biochemistry, chemistry, and medical sciences. The successful candidate will be provided with a competitive startup package and salary and will have access to outstanding research resources. The successful candidate will be expected to develop a vigorous externally funded research program and to participate in and develop courses at the undergraduate and graduate levels. Appointment is expected to be at the **ASSISTANT PROFESSOR** level, but outstanding **SENIOR-LEVEL CANDIDATES** will also be considered.

Applicants should send curriculum vitae, a statement of research (past, present, and planned) and teaching interests, representative publications and preprints, and arrange to have at least four letters of recommendation sent to: **Malcolm E. Winkler, Department of Biology, Indiana University Bloomington, Jordan Hall, Room 142, Bloomington, IN 47405.** Please address questions by **e-mail: mwinkler@bio.indiana.edu.** Review of applicants will begin as soon as possible and continue until the position is filled. *Indiana University is an Affirmative Action/Equal Opportunity Employer. Women, minority candidates, and couples are encouraged to apply.*

The Department of Biology of the University of Waterloo invites applications for two tenure-track positions at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level in animal physiology. Applicants must have a Ph.D. and postdoctoral experience and be prepared to establish an externally funded active research program. We are particularly interested in candidates using cellular and/or molecular approaches to explore an aspect of animal physiology. Duties include research, teaching at the undergraduate and graduate levels, and graduate student supervision. Candidates should be able to teach courses dealing with comparative animal physiology, reproductive animal physiology, endocrinology, and/or a specialized topics course within these disciplines. Salary will be commensurate with qualifications and experience. Applicants should send their curriculum vitae, the names of three individuals willing to furnish letters of reference, and an outline (one-to-two pages) of their proposed research program to: **Chair, Department of Biology, Faculty of Science, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, Canada N2L 3G1.**

The closing date for applications is November 15, 2004, with a start date after May 1, 2005. Additional information on the Department is available at **website: http://www.sci.uwaterloo.ca/biology.**

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority. The University of Waterloo encourages applications from all qualified individuals, including women, members of visible minorities, native peoples, and persons with disabilities.

POSITIONS OPEN

FACULTY POSITION
Peripheral Nerve Regeneration
Johns Hopkins University

The Departments of Orthopaedics and Neuroscience, Johns Hopkins University, invite applications from scientists with outstanding training and potential for a faculty position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level. The appointment will be based in the Peripheral Nerve Regeneration Laboratories of the Department of Orthopaedic Surgery, and will include participation in the activities of the Department of Neuroscience. We seek a scientist with a strong background in molecular neuroscience to investigate factors contributing to the specificity of peripheral axon development and regeneration. The successful candidate will be expected to collaborate with ongoing studies as well as establish and maintain a strong extramurally funded research program. Commitment to excellence in teaching at the graduate level is also required. Candidates should send their curriculum vitae, a statement of research goals, and contact information for three references to: **Thomas M. Brushart, M.D., Vice Chairman for Research, Johns Hopkins Orthopaedics, 601 N. Caroline Street, Baltimore, MD 21287. E-mail:tbrusha@jhmi.edu.**

Johns Hopkins offers competitive compensation and comprehensive benefits. As an Equal Opportunity Employer, we value diversity.

FACULTY POSITIONS IN BIOCHEMISTRY
University of California, San Diego

The Department of Chemistry and Biochemistry of University of California, San Diego (**website: http://chem.ucsd.edu**) invites applications for tenure-track/tenured faculty positions in biochemistry with a preference for candidates at the **ASSISTANT PROFESSOR** level. Candidates must have a Ph.D. and a demonstrated ability for creative research and teaching at the undergraduate and graduate levels. The Department will consider applicants in all areas of structural biology and biophysics for this position. Salary commensurate with qualifications and based on University of California pay scale. Candidates should send curriculum vitae, list of publications, reprints of up to five representative papers, and a summary of research plans to: **Chair, Biochemistry Search Committee 4-445S, University of California, San Diego, Department of Chemistry and Biochemistry, 9500 Gilman Drive–0332, La Jolla, CA 92093-0332.** Candidates should also arrange to have three letters of reference sent under separate cover. The deadline for applications is October 8, 2004, but until the position is filled, all applications received will be assured full consideration. *UCSD is an Equal Opportunity/Affirmative Action Employer with a strong institutional commitment to the achievement of diversity among its faculty and staff.*

ASSISTANT PROFESSOR
BIOLOGICAL CHEMISTRY
The David Geffen School of Medicine at UCLA

The Department of Biological Chemistry is seeking applications for a tenure-track faculty position in any area of biochemistry, or molecular, cell, and developmental biology. The position is at the level of Assistant Professor. The new faculty member will be expected to develop a strong and creative research program and to contribute to the teaching missions of the Department.

Selection of candidates will begin on November 1, 2004, and will continue until the position is filled. Applicants should send curriculum vitae and a summary of research interests and arrange for three letters of reference to be sent to:

Gregory S. Payne
Chair, Search Committee
c/o Karen Ronan
HHMI—UCLA
5-748 MRL
P.O. Box 951662
Los Angeles, CA 90095-1662

The University of California is an Equal Opportunity Employer.

THE STATE UNIVERSITY OF NEW JERSEY
RUTGERS

Director of the Institute for Advanced Materials and Devices

Rutgers University seeks an outstanding scientist and academic leader to become the first Director of the newly established Institute for Advanced Materials and Devices (IAMD). The primary mission of the Institute is to advance interdisciplinary research at the forefront of modern materials and devices. The IAMD is composed of approximately 75 faculty members drawn from various departments at the University, as well as several existing centers and laboratories. The Director will lead the coordination and integration of research and educational activities, develop new research and educational programs, seek new funding resources and serve as the Institute representative to internal and external constituencies.

Major thrusts of the IAMD include multi-functional materials and devices, nanoscience and technology, sensors, theory and computational modeling. The Institute will also interface with the strong life sciences programs at Rutgers and the Robert Wood Johnson Medical School. It will develop innovative programs that foster close working relationships with industry, as well as other academic and governmental laboratories.

The Director will report to the Executive Vice President for Academic Affairs and will qualify for appointment at the rank of professor, to be made jointly between appropriate departments in the Rutgers School of Engineering and the Faculty of Arts and Sciences-New Brunswick. The successful candidate should have an international reputation for outstanding scholarly research in an area within the scope of the IAMD, demonstrated leadership in materials and devices disciplines, a keen interest in and appreciation for education, a proven record of external funding, and the ability to interact effectively with administrators, faculty, students, industry representatives, and government officials. The appointment starting date is flexible. For more information about the IAMD and how to submit nominations and applications for the position, visit the Institute website **http://iamd.rutgers.edu**.

An Affirmative Action/Equal Opportunity Employer.

HOSPITAL FOR SPECIAL SURGERY

CORNELL UNIVERSITY
Joan and Sanford I. Weill Medical College

FACULTY POSITIONS
TISSUE ENGINEERING AND REPAIR

The Hospital for Special Surgery, in conjunction with the Weill Medical College of Cornell University, is undertaking a major new initiative in tissue engineering and regenerative medicine. We are recruiting a Program Director and tenure track research faculty for a new Program in Tissue Engineering and Repair. An endowed Chair in Tissue Engineering has been established at the Hospital to help meet this goal. The Hospital for Special Surgery is a leading hospital for musculoskeletal care, including treatment of arthritis and osteoporosis, spine and sports injuries, and cerebral palsy and spina bifida. The Research Division of the Hospital focuses on bone and cartilage physiology, tissue remodeling, inflammation, autoimmunity, and the pathophysiology of orthopedic and rheumatic diseases.

Faculty in the Tissue Engineering and Repair Program will be responsible for developing an innovative and competitive program of research in tissue engineering, repair, or mesenchymal stem cell biology that will interface with existing research Programs in Musculoskeletal Integrity, Autoimmunity and Inflammation, Arthritis and Tissue Degeneration, and Tissue Mechanics. There will be ample opportunity for collaboration with investigators at the adjacent Memorial Sloan-Kettering Cancer Center and Rockefeller University. It is expected that faculty will establish links with the Weill Medical College of Cornell University in New York City and/or Cornell University in Ithaca and participate in the academic programs of Cornell University. Faculty will hold a joint academic appointment at Cornell University.

HSS offers an extensive startup package and a highly competitive compensation package. Applicants should send a CV, a brief summary of research accomplishments and future objectives, and names and addresses of 3 references to: **Dr. Lionel Ivashkiv, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021**.

An Equal Opportunity/Affirmative Action Employer

Vanderbilt University Medical Center Faculty Positions in Cell and Developmental Biology

The Department of Cell and Developmental Biology invites applications for several tenure-track positions at the rank of Assistant/Associate Professor. Our department covers a broad range of topics including cellular biology, cellular signaling and trafficking, cell cycle regulation, neurobiology, developmental biology and stem cell biology. Cell biologists using cellular, molecular, biochemical and/or genetic approaches are encouraged to apply. Candidates must have a Ph.D. and/or M.D. degree, at least two years postdoctoral experience, a strong publication record and strong potential for extramural funding evident. Successful candidates will receive substantial start-up packages and be housed in newly constructed/renovated facilities. In addition, there is a strong institutional commitment to core facilities, graduate programs, and an interdisciplinary environment. Complete applications should include curriculum vitae, reprints of recent publications, a brief statement of present and future research plans, and three letters of recommendation, solicited by the applicant. Review of applications will begin **November 1, 2004**, and will continue until the positions are filled. Please send materials to: **Susan R. Wente, Ph.D., Professor and Chair, Department of Cell and Developmental Biology, Vanderbilt University Medical Center, 3120 Medical Research Building III, Nashville, TN 37232-8240.**

Vanderbilt University Medical Center is strongly committed to diversity in attracting faculty to fill these positions and is an Affirmative Action, Equal Opportunity Employer.

Theoretical Population Genetics UNIVERSITY OF CALIFORNIA, DAVIS

The Division of Biological Sciences, University of California, Davis invites applications and nominations for a position in the Section of Evolution and Ecology at the tenure-track ASSISTANT, ASSOCIATE or FULL PROFESSOR level. Candidates must have a Ph.D. (or equivalent) in the biological sciences or related fields. Candidates should have a strong record of research in theoretical population genetics, focusing on questions of interest to molecular population geneticists and molecular evolutionists. The successful candidate will be expected to teach an undergraduate course in introductory biology or evolution and to participate in the Core Course required of all first-year graduate students in the Population Biology Graduate Group.

Applicants should send the following items (as pdfs) to the Chair of the Theoretical Population Genetics Search Committee at **bashaneyfelt@ucdavis.edu**: curriculum vitae, description of current and projected research, summary of teaching interests and experience, and up to five publications. Applicants should also arrange to have three letters of recommendation sent by e-mail to **bashaneyfelt@ucdavis.edu**. Closing Date: Open until filled, but all application materials, including letters of recommendation, must be received by **November 1, 2004** to assure full consideration.

The University of California is an Equal Opportunity/Affirmative Action Employer with a strong institutional commitment to the development of a climate that supports equality of opportunity and respect for differences.

The Program in Molecular and Computational Biology at the University of Southern California invites applications for multiple tenure-track and tenured faculty positions. Our program continues to undergo significant expansion, including occupancy of a new research building in Spring 2005. We are an interactive multi-disciplinary faculty looking for colleagues who will enhance and complement our present research strengths. Extramural funding is expected.

We seek scientists who use modern molecular, genetic, or computational approaches to address fundamental molecular, cellular, or developmental processes. Investigators within the disciplines of **Evolutionary Biology, Neurobiology, Plant Biology, Systems Biology, or Bioinformatics** are especially encouraged to apply, although outstanding candidates in related fields will also be considered.

Review of applications will begin **October 15, 2004**. Please send a curriculum vitae, statement of research objectives, and three letters of recommendation to **msearch@usc.edu**. For additional information about our program, please visit **http://www.usc.edu/dept/LAS/biosci/mcb/**.

USC is an Affirmative Action/ Equal Opportunity Employer.

POPULATION/COMMUNITY ECOLOGIST
Department of Biology
Indiana University, Bloomington

The Department of Biology, Program in Ecology, Evolution, and Behavior invites applications for an Ecologist. We seek candidates with a conceptually driven research program utilizing theoretical, experimental, and/or field approaches to population and/or community ecology. The search is open to individuals working on any taxa, including microbes. Individuals complementing department strengths in interspecific interactions, disease ecology, and plant-microbe interactions are particularly encouraged to apply.

We invite candidates at the tenure-track **ASSISTANT PROFESSOR** level, but outstanding candidates at any level will be considered. The successful candidate will be provided with a competitive startup package and will be expected to establish a vigorous, externally funded research program and to participate in teaching undergraduate and graduate courses. For information about the Department of Biology and for links to the campus and the Bloomington community, see **website: http://www.bio.indiana.edu.**

Candidates should send curriculum vitae, a statement of research, and representative publications and should arrange to have three (or more) letters of recommendation sent to: **Jim Bever, Population/Community Ecology Search, Department of Biology, Indiana University, 1001 East Third Street, Bloomington, IN 47405-3700.** Review of applications will begin October 20, 2004, and will continue until suitable candidates are identified.

Indiana University is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are encouraged to apply.

The Department of Chemistry at The University of Toledo, home of the Ohio Macromolecular Crystallography Consortium, invites applications or nominations for a tenured or tenure-track **FACULTY POSITION** in macromolecular X-ray crystallography. This appointment will complement existing department strengths in structural biology and biological chemistry, and coincides with a major upgrade of the existing crystallography laboratory that has added a new high brilliance diffractometer, and a robotics crystallization and crystal imaging facility. The successful candidate will have a Ph.D. degree in chemistry or a related field, will be expected to have or to develop a vigorous, externally funded research program, and to have a commitment to excellence in teaching chemistry at both the undergraduate and graduate level. The University of Toledo offers competitive salaries and an excellent benefits package to its faculty. Further information on available faculty positions, the new research facilities for chemistry (Wolfe Hall), and the Instrumentation Center is available at **website: http://www.chem.utoledo.edu.** Senior applicants should send their curriculum vitae and a summary of research accomplishments; junior-level applicants should also send a research plan and arrange for three letters of recommendation to be sent to: **Chair, X-ray Crystallography Faculty Search Committee, Department of Chemistry MS 602, The University of Toledo, Toledo, OH 43606.** Review of applications will begin on October 1, 2004, and continue until a suitable candidate has been identified. *The Department encourages applications from minorities, women, and persons with disabilities. The University of Toledo is an Affirmative Action/Equal Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.*

FREELANCE SCIENCE EDITORS

Translations company seeks Editors to work on as-needed basis. Receive work via computer. Will edit documents translated from various languages into English, ensuring scientific accuracy and clarity. Education in biology, chemistry, or engineering. Editing experience not required, but need a keen eye for detail. Subjects include chemical patents, biotech, medical studies, and industrial standards. Send resume via **e-mail: phoenixtranslations@ev1.net.**

POSITIONS OPEN

USDA ARS Agricultural Research Service
www.ars.usda.gov

RESEARCH GENETICIST (PLANTS)
RESEARCH MOLECULAR BIOLOGIST (PLANTS)
GS-440/401-12/13/14

SALARY RANGE: $65,706 to $120,033

The U.S. Department of Agriculture, Agricultural Research Service, in Albany, California, is seeking a permanent, full-time Research Scientist. The successful applicant will conduct basic research in plant computational biology/plant genomics at the Plant Gene Expression Center, developing an independent program in plant genome research that enhances analysis and integration of data from the study of maize and other important agricultural genomes. The successful applicant will be considered for an **ADJUNCT FACULTY POSITION** at the Department of Plant and Microbial Biology, University of California, Berkeley. Candidates should have a Ph.D. degree and an excellent publication record. For application directions see **website: http://www.afm.ars.usda.gov/divisions/hrd/index.html**, announcement number ARS-X4W-295. Applications must be postmarked by September 17, 2004. For information about the Plant Gene Expression Center, see our **website at: http://www.pgec.usda.gov/** or **contact: Dr. Sarah Hake** at **e-mail: maizesh@nature.berkeley.edu.** *U.S. citizenship is required. USDA/ARS is an Equal Opportunity Employer and Provider.*

Roosevelt University is seeking an **ASSISTANT PROFESSOR** of biology for a tenure-track position to begin August 2005. The successful candidate will teach major courses in physiology, immunology, and general biology, and supervise undergraduate and Master's level students' research projects and internships. Applicants should have a Ph.D., strong knowledge of modern techniques and computational methods in biology, and a demonstrated commitment to teaching and undergraduate research. We especially encourage women and minority candidates to apply. Please send a letter of application, curriculum vitae, two-page statement of undergraduate teaching philosophy and research interests, and the names, telephone numbers, and e-mail addresses of at least three references to: **Cornelius Watson, Assistant Director of the School of Science and Mathematics, Roosevelt University, Chicago, IL 60605.** Screening will begin on November 15, 2004, and continue until the position is filled. **Telephone: 312-341-3678; fax: 312-341-4358; e-mail: cwatson@roosevelt.edu; website: http://www.roosevelt.edu.** *Roosevelt University is an institution dedicated to social justice that serves a diverse population of students with campuses in Chicago's South Loop suburban Schaumburg.*

FACULTY POSITION
Central Nervous System Drug Delivery/Vascular Biology

The Department of Pharmaceutical Sciences at Texas Tech University Health Sciences Center (TTUHSC) School of Pharmacy at Amarillo has an opening for a tenure-track faculty position at the **ASSISTANT/ASSOCIATE PROFESSOR** level to join an established group of researchers investigating brain drug delivery and vascular biology. Preference will be given to applicants with an extramurally funded program. In addition to maintaining their research program, the successful candidate will teach in the basic science components of the Pharm.D. and Ph.D. curriculum, and mentor graduate students. Startup packages and laboratory space are competitive. Submit curriculum vitae, a summary of research and teaching interests, and names and addresses of three references to: **Dr. Margaret Weis, Search Committee Chair, Texas Tech University School of Pharmacy, 1300 Coulter, Amarillo, TX 79106. E-mail: margaret.weis@ttuhsc.edu; fax: 806-356-4034.** *TTUHSC is an Equal Opportunity/Affirmative Action Institution. Minorities and Women are encouraged to apply.*

POSITIONS OPEN

ASSISTANT OR ASSOCIATE PROFESSOR TENURE TRACK
Cardiovascular Science–Pharmacology
Medical College of Georgia

We are seeking an individual with outstanding potential and accomplishments in cardiovascular research to complement and extend our current research strengths. We have established research programs in cardiac, endothelial, and vascular smooth muscle pharmacology, with specific interests in nitric oxide, oxidative stress, second messenger signal transduction mechanisms, hypertension, endothelial dysfunction, myocardial disease, diabetes, and cardiovascular ion channels. We encourage applicants with expertise in all areas of cardiovascular science, particularly in the factors that control cardiovascular dysfunction associated with diabetes and heart failure. Physician scientists are encouraged to apply. We offer a generous startup package and outstanding facilities are available for electron microscopy, cell imaging, microarray technology, genetically modified animals, primate research, and clinical collaborations. The successful applicant will participate in teaching programs for professional and graduate students. Please send curriculum vitae, summary of professional and research goals, and the names and addresses of three references to: **Richard E. White, Ph.D., Department of Pharmacology and Toxicology, Medical College of Georgia (MCG), Augusta, GA 30912-2300. E-mail: rwhite@mail.mcg.edu** and visit the Medical College of Georgia homepage (**website: http://www.mcg.edu**). Application review will begin October 2004. *MCG is an Equal Employment Opportunity/Affirmative Action Equal Access Employer.* PO# E000051148.

FACULTY POSITION
COMMUNITY ECOLOGY

The Department of Biology, Skidmore College, invites applications for a tenure-track **ASSISTANT PROFESSOR** position in community ecology, beginning in the fall of 2005. The successful candidate will complement the Department's existing strengths in population biology and will contribute to the environmental studies major. Applicants must have a Ph.D. in biology or a related discipline, teaching experience, a successful independent research program, and a primary interest in teaching undergraduates at a liberal arts and science institution; postdoctoral experience is preferred. Yearly course load will be selected from among a general ecology course for biology majors, an environmental biology course for environmental studies majors, and specialty courses in the candidate's area of expertise. Establishment of a strong research program that involves undergraduates is expected; excellent teaching and research facilities and support are available. Send curriculum vitae, statements of teaching and research interests, and three letters of recommendation to: **Corey R. Freeman-Gallant, Chair, Department of Biology, File #SI, Skidmore College, Saratoga Springs, NY 12866, Community Ecologist Search.** Review of applications will begin October 14, 2004.

Skidmore encourages applications from women and men of diverse racial, ethnic, and cultural backgrounds.

ASSISTANT PROFESSOR OF BIOLOGY

Saint Mary's College of California invites applicants for a tenure-track position to begin in the fall of 2005. Candidates' primary expertise must be in immunology or a closely related field. Teaching expectations will include courses in immunology, cell and molecular biology, and human physiology. Successful candidates must share the College's commitment to teaching excellence in a small liberal arts Catholic setting. An active program of scholarship involving participation by undergraduates will also be expected. Send curriculum vitae, teaching and research statement, and three letters of recommendation to: **Dr. Allan Hansell, Search Committee Chair, Department of Biology, Saint Mary's College of California, P.O. Box 4507, Moraga, CA 94575.** Review of applications will begin October 15, 2004.

AWARDS

Linus Pauling Institute Prize for Health Research

Call for Nominations

The Linus Pauling Institute Prize for Health Research is sponsored by the Linus Pauling Institute at Oregon State University (**http://lpi.oregonstate.edu**). The Prize consists of $50,000 and a medal, and is awarded biennially. The LPI functions from the basic premise that an optimum diet and a healthy lifestyle is the key to optimum health. The purpose of the Prize is to recognize innovation and excellence in research relating to the roles of micronutrients, vitamins, and phytochemicals in promoting health and preventing or treating disease; and the roles of oxidative/nitrative stress and antioxidants in human health and disease. The aim is to stimulate innovative research that enhances our knowledge of the role of diet and lifestyle in the primary and secondary prevention of disease, and the role of oxidative/nitrative stress in the causation of disease.

Procedure: The nominator should submit a nomination letter, two supporting letters solicited from his/her colleagues, and the candidate's up-to-date curriculum vitae. The candidate's research accomplishments in light of the purpose of the Prize should be amply described in the letters. The awardee must be present to accept the Prize and present a talk at the "Diet and Optimum Health" conference organized by LPI in Portland, Oregon, May 18-21, 2005. Nominations should be sent to: **Linus Pauling Institute, Attn: Barbara McVicar, Oregon State University, 571 Weniger Hall, Corvallis, OR 97331-6512**. Complete nomination materials must be received by **November 1, 2004**.

SYMPOSIA

MAYO CLINIC

MAYO CLINIC ANGIOGENESIS SYMPOSIUM - October 29-31, 2004
Rochester, Minnesota

ATTENTION POSTDOCTORAL FELLOWS, RESIDENTS, and STUDENTS!

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Vicki Bautch, Ph.D.
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Alex Adjel, M.D., Ph.D.
Vincent S. Rajkumar, M.D.
Kalpna Gupta, Ph.D.
and Others…

Abstract deadline extended to: September 24, 2004. CME credit is available to qualified physicians. Please see our website for registration, discounted travel, hotel, and abstract information: **http://mayoresearch.mayo.edu/mayo/research/angiogenesis.cfm.**

POSITIONS OPEN

UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

Faculty Positions in Infectious Diseases

The Division of Infectious Diseases in the Department of Medicine at the University of Texas Southwestern (UTSW) Medical Center at Dallas is seeking new faculty members at the Assistant Professor, Associate Professor, or Professor levels. Faculty will be expected to develop independent and externally funded independent research programs that focus on understanding the molecular pathogenesis of infectious diseases and/or host defense mechanisms. Preference will be given to applicants performing "cutting-edge" research on medically important pathogens, emerging pathogens, and/or agents of potential biothreat. Excellent opportunities exist for collaborations with faculty members in Infectious Diseases, the Department of Microbiology, and the Center for Immunology at UTSW and with the Regional Center of Excellence (RCE) for Biodefense and Emerging Infectious Diseases. UTSW is an outstanding scientific environment with established strengths in structural biology, biochemistry, molecular biology, genetics, and numerous other areas. Candidates will be expected to contribute to the teaching and research training of Infectious Diseases fellows. The position offers an attractive startup package and laboratory space. Candidates should have an M.D. and/or a Ph.D. degree with at least two years of postdoctoral experience and an outstanding publication record.

To apply, submit a C.V., three letters of reference, and a description of research interests to: **Dr. Beth Levine, Chief, Division of Infectious Diseases, c/o Renee Talley, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9113. E-mail: renee.talley@utsouthwestern.edu.**

UT Southwestern is an Equal Opportunity/ Affirmative Action Employer.

PROFESSOR OF BIOLOGY, CHEMISTRY, OR PHYSICS, AND DEPARTMENT CHAIR JOINT SCIENCE DEPARTMENT THE CLAREMONT COLLEGES

Claremont McKenna, Pitzer and Scripps Colleges, selective liberal arts colleges in the Claremont Colleges Consortium, seek an accomplished teacher-scholar to serve as professor and chair of our integrative Joint Science Department (**www.jsd.claremont.edu**). The successful candidate will be qualified to hold a senior tenured faculty rank in biology, chemistry or physics. He/she will teach and do productive research in her/his field and lead the Department for a three-year term. Teaching responsibilities will be reduced by 60% to allow for administrative duties. The Department has 25 faculty in life and physical sciences and offers majors in the three disciplines, as well as in a variety of interdisciplinary areas. In addition, the Department serves the needs of nonscience majors with a series of specially designed courses. Faculty are extensively involved in research, particularly with undergraduates. The Department is housed in a single facility opened in 1992 and expanded in 2000, with excellent facilities for research and teaching. Adequate startup funds for the new chair's research will be provided. The Department Chair reports to the Dean of Faculty, develops budgets, manages staffing, and provides leadership in assessing departmental needs and assisting in fundraising to meet those needs.

Applications will be reviewed beginning **September 30, 2004,** and continue until the position is filled. Please send a letter of application describing your interest in the position, a statement of teaching interests and philosophy, curriculum vitae, and a statement of research accomplishments and goals, and have three letters of reference sent to: **Dr. James Higdon, Professor of Physics, Keck Science Center, 925 N. Mills Ave., Claremont, CA 91711; jhigdon@jsd.claremont.edu.**

In a continuing effort to enrich our academic environment and provide equal educational and employment opportunities, The Claremont Colleges actively encourage applications from women and members of historically underrepresented groups in higher education.

POSITIONS OPEN

PROFESSOR AND HEAD
Department of Chemistry and Molecular Biology

Nominations and applications are invited for the position of Professor and Head of the Department of Chemistry and Molecular Biology at North Dakota State University (NDSU). All areas of these disciplines will be considered. The appointment begins on or about May 2005. Applicants should have a Doctorate in chemistry, biochemistry, or related field, and an outstanding record in research and undergraduate/graduate teaching appropriate to appointment at the rank of **PROFESSOR** with tenure; leadership and administrative skills; have an extramurally funded nationally competitive research program and the ability to implement it at NDSU; demonstrated ability to work effectively with colleagues in an academic setting; and have effective oral and written communication skills. The Department of Chemistry and Molecular Biology (**website: http://www.chem.ndsu.nodak.edu**) is a Ph.D.-granting unit of the College of Science and Mathematics, which enrolls 2,000 of the University's 12,000 students. The Department has 18 faculty, with research expenditures averaging $3 million per year. Several lecturers assist with teaching and Ph.D.-level research specialists staff all the major instrumentation facilities (molecular modeling, nuclear magnetic resonance, mass spectrometry, X-ray crystallography). The Department averages 90 undergraduate majors, 35 graduate students, and 20 postdoctoral researchers. The University is expanding its research enterprise, particularly through its new Research and Technology Park, graduate fellowships, and research centers. The Department is playing an integral role in this growth. NDSU is located in Fargo (**website: http://ci.fargo.nd.us**), the largest city in North Dakota, which is often cited for its high quality of life. Candidates should submit a letter of application, curriculum vitae outlining qualifications in research, teaching, and leadership, a discussion of the research program that would be conducted at NDSU, and contact information for five references to: **Professor Gregory R. Cook, North Dakota State University, Department of Chemistry and Molecular Biology, P.O. Box 5516, Fargo, ND 58105-5516. E-mail: gregory.cook@ndsu.nodak.edu.** Review of applications will begin on November 15, 2004, and continue until the position is filled. *NDSU is an Equal Opportunity Institution.*

DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
National Institute of Diabetes and Digestive and Kidney Diseases
Laboratory of Molecular Biology

National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health (NIH), is recruiting for a **STAFF SCIENTIST** position in computational protein crystallography. The position entails responsibility for the system management of a cluster of computers that serves the computational needs of three protein crystallographic groups consisting of about 12 scientists, as well as engaging in the research program of the Section on Molecular Structure, which involves HIV and immunology. Qualifications should include experience with protein crystallography and computer system management, including operating systems UNIX and LINUX. Salary commensurate with experience and a full Civil Service package of benefits (including retirement, health, life and long-term care insurance, thrift savings plan participation, etc.) is available. Applications, together with three references, should be addressed to:

David R. Davies
Building 5, Room 338
NIH, Department of Health and Human Services
5 Center Drive, MSC 0560
Bethesda, MD 20892-0560
E-mail: david.davies@nih.gov

The deadline for applications is November 14, 2004. *DHHS/NIH are Equal Opportunity Employers.*

POSITIONS OPEN

ASSISTANT PROFESSOR of Biochemistry/Molecular Biology: The Department of Biological Sciences at Michigan Technological University invites applications for a tenure-track position (fall 2005) in biochemistry and molecular biology. The successful applicant must have postdoctoral experience, a demonstrated expertise in the use of current biochemical and molecular techniques, and will be expected to establish an extramurally funded research program, supervise graduate students, and interact with departmental faculty in research areas ranging from microbial to plant/animal systems. The position requires a commitment to undergraduate and graduate instruction in biochemistry and molecular biology. Additional information can be obtained at the departmental **website: http://www.bio.mtu.edu/.** Applicants should submit curriculum vitae, including a statement of research interests, teaching philosophy, and three letters of recommendation to: **Chair, Search Committee, Department of Biological Sciences, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931. Telephone: 906-487-2025; e-mail: committeechair@mtu.edu.** Review of applications will begin on October 29, 2004, and will continue until the position is filled. *Michigan Technological University is an Equal Opportunity Educational Institution/Equal Opportunity Employer.*

Tufts University, Department of Biomedical Engineering, is seeking candidates for a faculty appointment at the **ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR** level. Rank will be determined by experience and accomplishments. Preference will be given to candidates whose research interests involve regenerative medicine systems, and complement the Department's strengths in biomedical imaging and instrumentation. The successful candidate will join an active Ph.D.-granting department and must demonstrate the potential to develop an outstanding, internationally recognized research program, excel in teaching, and develop strong inter-school collaborations. Teaching responsibilities include graduate and undergraduate courses. A Doctorate is required and postdoctoral experience is desirable. Additional information about the Department can be found at **website: http://ase.tufts.edu/biomedical/.** Interested applicants should send curriculum vitae, cover letter, research plan, and names of three references to: **Faculty Search Committee, Department of Biomedical Engineering, Tufts University, 4 Colby Street, Medford, MA 02155.** Evaluation of candidates will begin at the end of October 2004 and continue until the position is filled. *Tufts University is an Affirmative Action/Equal Opportunity Employer. We are committed to increasing the diversity of our faculty. Applications from women and members of underrepresented groups are strongly encouraged.*

INVERTEBRATE ZOOLOGIST

The Department of Biology, College of Charleston, invites applications for a tenure-track position in invertebrate zoology at the **ASSISTANT PROFESSOR** level. Candidates must possess a Ph.D., a strong commitment to teaching, and an active research program with the potential for undergraduate involvement. Teaching responsibilities include a lecture and laboratory course in invertebrate zoology. We are particularly interested in applicants who break disciplinary boundaries (e.g., biomechanics, larval biology, marine genomics). The College of Charleston is a public liberal arts and sciences institution of 10,000 students. The College's primary aims are teaching and research excellence. In addition to its undergraduate programs, the Department offers M.S. degrees in marine biology and environmental studies. Information about the Biology Department can be found at **website: http://www.cofc.edu/~biology/.** Applicants should submit curriculum vitae, statements of teaching philosophy and research interests, reprints of recent publications, and three letters of reference by 1 November 2004 to: **Chair, Department of Biology, College of Charleston, Charleston, SC 29424.** *The College of Charleston is an Equal Opportunity/Affirmative Action Employer and encourages the applications of qualified women and minorities.*

POSITIONS OPEN

TENURE-TRACK POSITIONS
Biology Department

The Department of Biology invites applications for tenure-track positions in the following areas to enhance rapidly expanding programs.

(1) **ASSISTANT/ASSOCIATE PROFESSOR.** Freshwater wetland ecology within a Ph.D. program in aquatic resources. We seek a candidate broadly trained with interdisciplinary abilities and experience that bridge basic and applied scientific and management problems at the community, ecosystem, or landscape scales. Quantitative and spatial analysis skills are desirable. Note #2005-24 on application.

(2) **ASSISTANT PROFESSOR.** Wildlife ecology. We seek a candidate with expertise in mammalian or avian ecology, especially related to distance sampling of wildlife populations and geographic information system application to such data. Note #2005-25 on application.

Individuals are to establish extramurally funded research programs, train graduate students, and teach at the undergraduate and graduate levels. Qualified applicants are invited to submit curriculum vitae, copies of up to five publications, statements of teaching and research interests, and a list of four references including telephone numbers and e-mail addresses to: **Joseph Koke, Interim Chair, Biology Department, Texas State University–San Marcos, San Marcos, TX 78666.** Review of applications begins on 30 October 2004 and continues until successful candidates are found. San Marcos is located in the scenic Texas Hill Country midway between Austin and San Antonio. Texas State University owns and manages the headwaters of the spring-fed San Marcos River and associated ecosystems. For additional information about our Department, see **website: http://www.bio.txstate.edu.** *Texas State University is an Equal Opportunity Employer.*

Department of Biology. DePauw University. **MICROBIOLOGIST/MOLECULAR BIOLOGIST.** Tenure-track position beginning August 2005. Rank and salary commensurate with experience. Ph.D. preferred, all but dissertation required. Commitment to undergraduate teaching in liberal arts setting essential. Teaching responsibilities include: laboratory courses in microbiology, molecular biology, and cell biology. Successful candidate will be expected to conduct summer research projects with undergraduate students. For information about the Department, visit **website: http://www.depauw.edu/acad/biology/.** DePauw has exceptional programs for supporting its faculty members, including a pre-tenure leave and funding for professional and curriculum development activities (see **website: http://www.depauw.edu/admin/acadaffairs/facdev.htm**). Submit letter of application, curriculum vitae, three letters of recommendation, transcripts, statements of teaching interests and philosophy, research interests, and evidence of teaching effectiveness to: **Henning Schneider, Search Chair, Department of Biology, DePauw University, Greencastle, IN 46135.** Review of applications begins October 1, 2004, and continues until position is filled. *DePauw University is an Affirmative Action/Equal Opportunity Employer. Women and members of underrepresented groups are encouraged to apply.*

ANNOUNCEMENTS

STAGLIN FAMILY MUSIC FESTIVAL SCHIZOPHRENIA RESEARCH AWARD NARSAD

National Alliance for Research on Schizophrenia and Depression (NARSAD) announces a new Schizophrenia Research Award of $250,000 for one investigator with M.D., Ph.D., or doctoral degree and postdoctoral training applicable to schizophrenia, at Assistant or Associate Professor level. Nomination from dean, chair, or head of scientific program from employing university required. Deadline: December 3, 2004. Start date: July 1, 2005. See **website: http://www.narsad.org** or **telephone: 516-829-5576.**

National Institute of Diabetes & Digestive & Kidney Diseases

Call for Innovative Ideas to Advance Prevention, Treatment and Cure of Type 1 Diabetes

Background: The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), leads a Special Statutory Funding Program ($150 million/year) for Type 1 Diabetes Research, on behalf of the Secretary of the Department of Health and Human Services. The special program supports research to pursue compelling opportunities in type 1 diabetes research (more information on the program can be found at: **http://www.niddk.nih.gov/fund/diabetesspecialfunds/**). The program is framed around six broad, scientific goals:

Goal I: Identify the Genetic and Environmental Causes of Type 1 Diabetes
Goal II: Prevent or Reverse Type 1 Diabetes
Goal III: Develop Cell Replacement Therapy
Goal IV: Prevent or Reduce Hypoglycemia
Goal V: Prevent or Reduce the Complications of Type 1 Diabetes
Goal VI: Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes

Call to Identify New and Emerging Scientific Opportunities: The NIDDK invites submission of innovative ideas and approaches toward accelerating progress and overcoming research barriers to the prevention, treatment and cure of type 1 diabetes and its complications. There is significant flexibility in the use of these funds; opportunities could be pursued through solicitations for traditional research grants or through mechanisms to support larger collaborative efforts. Suggestions should focus on identifying the opportunity and approaches, technology and expertise useful for its development rather than on a funding instrument or solicitation design. Suggestions that will involve creative scientists and scientific communities not currently working on type 1 diabetes, and with the potential to contribute to prevention, treatment and cure of type 1 diabetes, are particularly encouraged.

Use of Submissions: Submissions will be considered by NIH and CDC scientists and by an advisory panel to be convened by NIDDK as part of the process guiding use of the Special Statutory Funding Program for Type 1 Diabetes Research. Therefore, submissions will not be confidential, although they may be anonymous. New research solicitations and/or program enhancements may be developed to address ideas submitted; however, there is no funding associated with this announcement.

Submitting an idea: Please describe your suggested opportunity or approach including: (1) how it could potentially have a major, positive impact on one or more of the six goals above, and/or (2) the current research barriers it could help overcome. You are welcome to submit more than one idea, but please limit the description of each to one page in length. For hard-copy submission, please mail or FAX your submission(s) to: **Topics Regarding Type 1 Diabetes Research Opportunities, NIH/NIDDK/OSPPA, 9000 Rockville Pike, Building 31, Room 9A05, Bethesda, MD 20892-2560, Attn.: Mary C. Hanlon, Ph.D.; Phone: 301-496-6623; FAX: 301-480-6741.** For electronic submissions, please visit the following website: **http://www.niddk.nih.gov/forms/T1D-Opportunity.htm**.

Deadline: For full consideration, submissions are due by **Friday, October 22, 2004**.

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